EAHRC
EAST AFRICAN HEALTH RESEARCH COMMISSION
Research for Health and Prosperity

ORIGINAL ARTICLE

Isoniazid and Rifampicin Tuberculosis Drug Resistance in HIV Endemic Region of Western Kenya

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ABSTRACT

Background: Tuberculosis drug resistance is often associated with inadequate anti-tuberculosis treatment regimen resulting to mutations that confers resistance to anti-tuberculosis agents. This is aggravated by synergetic relationship between Tuberculosis and HIV (Human Immunodeficiency Virus). Over 25% of Global Tuberculosis deaths occur in Africa and Kenya is one of the 30 high burden countries that together account for more than 80% of the world's TB cases. According to World Health Organization, in 2018, Multi drug resistant Tuberculosis prevalence in Kenya was 1.3% in new cases and 4.4% in retreatment cases. Kisumu County recorded the second highest HIV prevalence at 18.6% against the national prevalence of 4.5% in 2020. The extent of regional burden of DR-TB and HIV co-infection has not been exactly well-defined in Western Kenya.

Methods: This was a prospective cross sectional study that aimed to explore the association between Tuberculosis drug resistance and HIV status among new and previously treated pulmonary tuberculosis cases in Kisumu County, Kenya. Tuberculosis clinical suspects were recruited into the study and classified as HIV positive or negative based on their clinical data. Sputum samples from tuberculosis clinical suspects were subjected to fluorescent microscopy, phenotypic culture and line probe assay.

Results: Out of a sample of 256, response rate was 216 of which HIV positive cases were 119(55.1%) and negative were 97 (44.9%). The study found that out of 11 that were phenotypic Isoniazid resistance 8(6.7%) were from HIV positive cases while 3 (3.2%) were from HIV negative cases. Phenotypic rifampicin resistance among the HIV positive were 8 (6.7%) while HIV negative were 2 (2.1%). All the 2(1.7%) MDR cases were from HIV positive participants. The study found out that HIV status and Tuberculosis cases were significantly associated at p<.05. HIV positive cases were more likely associated with retreatment cases (OR=1.4,95CI:1.00-1.90) compared to new cases.
The study found out that the common mutant probe among the HIV positive was katG MUT1 4(2.6%), while mutant probes among the HIV negative were in hA MUT1 1(0.7%), katG MUT1 1(0.7%) and roB MUT2A 1(0.7%). Wild type gene deletion among the HIV positive cases were observed in probes katG WT 3(2.1%), roB WT7, katG WT 1(0.7%) while wild type gene deletion among the HIV negative cases were inhA WT1 1(0.7%), in hA WT1/inhAWT2 1(0.7%), katG WT 1(0.7%).

Conclusion: Interventions specific to HIV-endemic areas are urgently needed to block tuberculosis drug resistance transmission. Development and improvement of the efficacy of interventions will require a greater understanding of the transmission of multidrug-resistant tuberculosis in HIV-endemic settings like Kisumu County, Western Kenya.

BACKGROUND

The emergence and spread of Drug-resistant TB (DR TB) and Multidrug-resistant TB (MDR TB) have become a global health problem positioning tuberculosis as one of the top 3 infectious disease killers. Globally, the prevalence of drug-resistant tuberculosis (DR-TB) has increased substantially in the past 2 decades making Tuberculosis the leading cause of death among people living with HIV infection, accounting for approximately 40% of deaths among this population.[1,2] Tuberculosis has been associated with morbidity and mortality, especially in poor resource settings and is often the first indicator of HIV infection.[3] According to the World Health Organization (WHO), there were an estimated 9.0 million incidence cases of TB globally in 2018.[3] More than half of these cases (56%) were in South-East Asia and Western Pacific regions, while 29% were in the African region.[4] Additionally, WHO estimated that, 8.6% (7.4% to 10%) of 10 million (range, 9 to 11.1 million) incident cases with active TB were also coinfected with HIV in 2018,[5] while rifampicin-resistant (RR) or multidrug-resistant (MDR-TB) occurred among 3.6% new cases, 18% previously treated and 5.6% among all TB cases. Inappropriate use of antibiotics in treatment of drug-

susceptible patients, sub-optimal treatment regimens and failure to complete treatment in drug susceptible patients leads to drug resistance.[6] This has therefore resulted in high treatment failures and death rates due to the complexities in diagnosis and treatment.[7] Kenya is among the 14 countries globally that are in all the 3 lists of high burden countries (HBC) for TB, TB/HIV and MDR-TB and the fifth highest burden country in Africa.[8] The estimated incident for TB in Kenya is 348/100,000 population, translating to about 169,000 TB cases occurring annually, the mortality rate (excluding HIV+TB) is 60/100,000 of the population.[9] According to WHO, in 2018, the MDR-TB prevalence in Kenya was 1.3% in new cases and 4.4% in retreatment cases.[10] Tuberculosis drug resistance affects all age groups, but has greatest toll in the most productive age group of 15 to 44 years and the major factor responsible for the large TB disease burden in Kenya is the concurrent HIV epidemic.[11]

A study done on the prevalence and detection of drug resistant mutations in Mycobacterium tuberculosis among drug naïve patients in Nairobi between 2015 and 2017 showed that out of the 132 study participants screened for tuberculosis, only 2 showed resistance associated with first line and second-line anti TB drugs.[12] Of the 2 patients that had resistance to first and second line, one showed resistance to *isoniazid*, while the other depicted an MDR-TB case resistant to both *rifampicin* and *isoniazid*.[12] Out of a total of 132 patients that were tested for resistance to second-line antituberculosis agents, one showed cross-resistance to *kanamycin*, *amikacin*, and *capreomycin*.[12] The same study showed that, MDR-TB cases had an additional second-line anti tuberculosis drug resistance while mono-resistant cases had no additional second-line drug resistance.[12] In Western Kenya, anti-tuberculosis drug resistance is an emerging health problem especially in Kisumu County where cases of HIV and TB co-infection are predominant.[13] Human Immunodeficiency Virus and TB are synergistic with HIV increasing the incidence of TB and TB associated with increased mortality among people living with HIV, and as an indicator of Acquired Immunodeficiency Syndrome (AIDS) defining illness. Other studies show that the risk of TB infection is 16 to 27 times greater in People Living with HIV (PLHIV) than in the general population.[12] According to the 2019 report from Kenya National Tuberculosis, Leprosy and Lung Disease program, Kisumu County had the third highest TB co-infection rate in Kenya at 59% after, Homa bay 64% and Siaya 63% which is way above the national co-infection rate of 28%.[11] The report further states that the TB prevalence rate in Kisumu is 379 out of 100,000 people which is higher than the average National TB prevalence of 223. According to the Ministry of Health report on Kenya HIV Estimates, Kisumu County has the second highest HIV prevalence of 18.6% after Homa bay 20.2% and Siaya 17.8% against the national prevalence rate of 4.5%.[11] This high prevalence possess a greater challenge in Tuberculosis control and drug resistance surveillance in Kisumu County as HIV is more likely associated with TB.[10]

The emergence and transmission of drug-resistant tuberculosis epidemic is a threat to regional control of Tuberculosis. Globally, various studies show that, HIV has a profound effect on the progression of tuberculosis hence elevated transmission. The extent of regional DR-TB and HIV co-infection burden has not been well-defined and data on the relationship between HIV status and Tuberculosis drug resistance specific to HIV-endemic areas like Kisumu County in western Kenya are urgently needed to bridge tuberculosis transmission gaps and modulate continued spread of drug resistance.

The Genus Mycobacterium

The *genus Mycobacteria* belong to the family *Mycobacteriaceae* which are characterised by elevated lipid content, most notably a high level of waxes called *mycolic* acids.[14] These *mycolic* acids are responsible for the organism being resistant to decolonisation by acid alcohols hence are referred to as being acid-fast.[14] The importance of the *Mycobacteria* cannot be overemphasised and continued study is required to further delineate the role of these organisms in disease. Tuberculosis is a disease caused by the bacillus *Mycobacterium* tuberculosis species which typically affects the lungs (pulmonary TB) but can also affect other sites (extra pulmonary TB).[15] Tuberculosis is one of the top 10 causes of death worldwide and the leading cause of death from a single Infectious agent (ranking above HIV/AIDS).[16] Globally, an estimated 10.0 million people got infected with TB in 2018 of which there were an estimated 1.2 million TB deaths among HIV-negative people and an additional 251000 deaths among HIV positive people.[17] Studies shows that Tuberculosis affects all sexes across all age groups, however the highest burden is in men of 15 years and above who account for 57% of all TB cases in 2018. By comparison, women accounted for 32% and children (aged <15 years) for 11%. Among all TB cases, 8.6% were people living with HIV (PLHIV). Geographically, most TB cases in 2018 were in the WHO regions of South-East Asia (44%), Africa (24%) and the Western Pacific (18%), with smaller percentages in the Eastern Mediterranean (8%), America (3%) and Europe (3%).[18] Drug-resistant TB continues to be a public health threat. In 2018, there were about half a million new cases of *rifampicin*-resistant TB (of which 78% had multidrug resistant TB).[19] The 3 countries with the largest share of the global burden were India (27%), China (14%) and the Russian Federation (9%).[10] Globally, 3.4% of new TB cases and 18% of previously treated cases had multidrug resistant TB or *rifampicin*-resistant TB (MDR/RR-TB), with the highest proportions (>50% in previously treated cases) in countries of the former Soviet Union.[10] In a study that was conducted by Nduba and others, Kenya ranks 10th globally among the TB burden countries. The high prevalence of HIV is a major contributing factor to high TB incidence.[20]

Anti TB Drugs

Tuberculosis can be managed effectively by first line anti-tuberculosis agents. However, this first line therapy may fail to cure Tuberculosis for varied reasons. Naivety among patients and continued spread of the Mycobacterium bacilli contribute to the emergence of drug resistant strains. The emergence of multidrug resistant TB (MDR-TB), is of great concern because it requires the use of second-line drugs that are difficult to procure, much more toxic and expensive than First Line Drugs (FLDs).[10]

Thus, the detection and treatment of drug susceptible or

single drug resistant TB is an important strategy for preventing the emergence of MDR-TB.[19] Extensively drug-resistant (XDR-TB) which are MDR strains that are resistant to *isoniazid rifampicin* and any *fluoroquinolones*, and to at least one of the 3 second-line anti-tuberculosis injectable drugs—i.e., *capreomycin*, *kanamycin*, and *amikacin* have also been reported.[8]

Kenya has adopted the current WHO recommendations on treatment and care for drug-resistant TB which stipulates that in patients with confirmed rifampicin-susceptible, isoniazid-resistant tuberculosis (Hr-TB), treatment with *rifampicin*, *ethambutol*, *pyrazinamide* and *levofloxacin* is recommended for a duration of 6 months while in patients with confirmed rifampicin-susceptible, isoniazid-resistant tuberculosis, it is not recommended to add *streptomycin* or other injectable agents to the treatment regimen.[11] A shorter all-oral *bedaquiline*-containing regimen of 9 to 12 months' duration is recommended in eligible patients with confirmed multidrug- or rifampicin-resistant tuberculosis (MDR/RR-TB) who have not been exposed to treatment with second-line TB medicines used in this regimen for more than 1 month, and in whom resistance to *fluoroquinolones* has been excluded.[21] Patients who are at high risk of DR TB are identified and are prioritised in receiving further Drug Susceptibility Testing (DST) beyond GeneXpert in the TB reference laboratories. This test includes first line (FL) and second line (SL) probe assay (LPA), culture and phenotypic DST.

Mycobacteria Tuberculosis Drug Resistance and HIV

Drug-resistant Tuberculosis is considered a potential obstacle for elimination of TB globally. Surveillance reports show that 12 million people living with HIV are co-infected with TB. Sub Saharan Africa bears the global burden with 70% of all the cases.[5] Co-infection of HIV and MDR-TB complicates Tuberculosis control and management. A number of studies have documented increased mortality among patients co-infected with MDR-TB and HIV. This co-infection has been equally responsible for Extensively drug-resistant TB (XDR-TB) outbreaks.[5] Better outcomes with decreased mortality have been described with concomitant treatment for both anti TB and anti HIV drugs.[5]

The extent of global problem of DR-TB and HIV co-infection has not been well-defined. Some of the reasons for the lack of these data are; HIV testing and TB drug resistance testing are not adequately assessed through surveillance. Surveillance from different studies from different geographical settings have shown discordant associations due to heterogeneity in setting, demographic profile, methodology and analysis of data.[22] According to WHO, 24 countries reported data on MDR-TB stratified by HIV status. The findings show that there was heterogeneity in geographic distribution with the majority confined to high-risk groups, even in countries showing a high prevalence of MDR-TB along with an emerging HIV epidemic. Only 11 countries, majority from Eastern European and Central Asian regions reported strong associations between HIV and drug resistance.[16] There are several epidemiological reasons that M/XDR-TB may be associated with HIV. The reasons suggested are rapid progression of disease due to harbouring of DR strains, particularly in the immune compromised compared to - immunocompetent state; drug mal absorption of anti-TB drugs, such as *Randethambutol* (E), leading to drug resistance and treatment failure; early reactivation of an infection due to increased vulnerability in an immune compromised state acquired from community or institutional transmission; direct contact with DR-TB cases, suggesting primary or transmitted resistance.[23] Resistance that has been acquired can be reduced by adhering to optimised therapy, whereas the control and management of primary resistance requires interventions to block the dynamics of transmission.[24] Understanding the importance of attained and primary resistance is important in implementing TB control policies especially in HIV-endemic settings, where high incidence of primary resistance have been reported.[18] Infection with HIV can influence tuberculosis drug-resistant in many ways, including the length and magnitude of infectiousness, the duration of exposure, and the vulnerability of the exposed population.[25,26]

Kenya is among the 14 countries globally that are in all the three lists of high burdened countries for TB, TB/HIV and MDR-TB and the fifth highest burdened in Africa.[22] According to the 2019 report from Kenya National Tuberculosis, Leprosy and Lung Disease program, Kisumu County was among counties with the highest TB co-infection rate in Kenya (59%) after, Homabay (64%) and Siaya (63%) which is way above the National co infection rate of 28%.[11] The report further states that the TB prevalence rate in Kisumu is at 379 out of 100,000 people, this is higher than the average National TB prevalence of 223 per 100,000 people. Although the development of drug resistant TB strains and subsequent treatment failure is a common clinical scenario in Kenya, information about TB drug resistance among HIV infected population is scanty, especially in HIV predominant regions like Kisumu County.[8] Different studies in Kenya have shown that people living with HIV are more associated with tuberculosis, however, data on the relationship between tuberculosis drug resistance and HIV status are heterogeneous nationally. As such and given the existing gap, there was need to determine the association between tuberculosis drug resistance and HIV status in HIV endemic region of Kisumu County, Western Kenya.

MATERIALS AND METHODS

Study Site

This study was carried out in Kisumu County, located in Western Kenya. The County lies between latitude 0^0 20'S and 0^0 50'S and longitudes 33^0 20'E and 35^0 20'E. It is bordered by various counties as follows; Kericho lies to the East, Nandi to the North East, Homa Bay to the South, Vihiga to the North West, Siaya County to the West, and delimited by Lake Victoria, the second largest freshwater lake in the World. Kisumu covers approximately 567km^2 on water and 2086km^2 of land area, representing 0.36% of the total land area of Kenya's 580,367km^2.[27]

The County has a total population of 1,153,343; 489,392 between 0 to 15 years and 663,951 being 15 years or above.[28] Administratively, the County has 7 Sub-counties namely: Kisumu East, Kisumu West, Kisumu Central, Nyando, Muhoroni, Nyakach, and Seme. The health care tier system in Kisumu County consists of level 1 (commu-

nity facilities), level 2 (Dispensaries), level 3 (Health centres), level 4 (county hospitals) and level 5 (County referral hospital). This study recruited patient from all health facilities handling Tuberculosis patient within the county. The HIV prevalence between 15 to 49 years is 16.3% (male 15%, female 17%) with an average prevalence of 18.6% against the national prevalence of 4.5%. Malaria remains a major health problem with a prevalence estimated at 27%.[27] The TB prevalence rate in Kisumu is 379 out of 100,000 people which is higher than the average National TB prevalence of 223 and TB-HIV co-infection rate of 59%.[28]

Study Design

Hospital and laboratory based descriptive cross sectional study design was done on Tuberculosis patients attending TB clinics and hospital facilities within Kisumu County. The study included all clinically suspected TB patients. This study was conducted between November 2020 and October 2021 to understand the magnitude of first line Tuberculosis drug resistance burden among HIV cases from Kisumu County, Kenya.

Sampling Technique

This study employed 100 percent sample of all clinically suspected Tuberculosis patients attending various health facilities within Kisumu County. Tuberculosis clinical suspects were recruited into the study and classified as HIV positive or negative based on their clinical data. Sputum samples from Tuberculosis clinical suspects were subjected to fluorescent microscopy, BACTEC™ MGIT™ 960 system (MGIT) (Becton Dickinson (BD) Bioscience, Erebodegem, Belgium) and Geno Type MTBDR*plus* (Hain Life Science GmbH, Nehren, Germany). Saturated sampling was preferred in this study because TB Clinics and Hospital facilities within Kisumu County were quite few.

Inclusion Criteria

The patients enrolled in the study had to be clinically presenting as a TB case as per the Kenyan Ministry of health case definition for Tuberculosis, and capable of expectorating sputum for study purposes. Informed consent (or parental permission), after demonstrating their understanding formed the basis for recruitment. For children under age 12, parental consent was sought, and both assent and parental consent was sought for participants ≥ 12 to <18 years of age, assent required signing of a consent form.

Exclusion Criteria

1. New patients who had started TB treatment more than one week before the study were excluded from enrolment. This is because patients who submit sputum samples after starting treatment, and in whom a positive sputum smear is observed, are more likely to be harbouring drug resistant strains, thus introducing bias. Additionally, a significant proportion of cultures would fail to grow in patients on treatment.

2. Patients who were unable to provide adequate sputum specimen for testing.

Sample Size

The sample size was calculated based on a sampling method recommended by WHO for drug resistance survey in tuberculosis.[29] The sample was determined by taking the prevalence of rifampicin resistance of 1.3% from previous year, desired precision of 0.5%, a 95% Confidence Interval and non-response rate of 15%. The calculated sample size needs to be increased by 15 to 20% to account for expected losses. Losses include patients diagnosed as smear positive who do not return to the diagnostic centres or do not produce an adequate sample for the survey and patients whose susceptibility testing does not give interpretable results,[29] (WHO Guidelines for drug resistance Survey).

$$n = N * z^2 * (1 - g) / (N - 1) * d^2g + z2 * (1 - g)$$

Where:

N = total number of new sputum smear positive pulmonary patients registered in the selected sentinel sites during one year;

z = z-value (from the standard normal distribution) that corresponds to the desired Confidence Level (narrowing the Confidence Interval from 95% to 90% will result in some reductions in sample size;

if Confidence Interval =90%, z= 1.65);

d = absolute precision (as a decimal, e.g. 0.01 or 0.02 meaning to err within 1 or 2% of the true proportion);

g = previous estimate of proportion of new cases with rifampicin resistance * (1 + anticipated change of previous estimate). The anticipated change can be considered as the change that the sentinel system should be able to detect. This change is expressed as a decimal, with a negative sign if a decrease is anticipated or a positive sign if an increase is anticipated. For example, a 40% decrease from the previous estimate would be expressed as an anticipated change of -0.4; thus g = earlier estimate * (1 - 0.4) = previous estimate * 0.6

The sample size hence was =256

Where, N=223 per 100,000, d= 0.05%, Z=1.96, P=1.3%

Sample Collection Transport and Storage

Study participants who met the minimum inclusion criteria were recruited into the study. They were then given sputum cups by the clinician or laboratory personnel in the recruiting facility to have their sputum samples taken. A pipette drop from the sample was used to perform bacteriology so as to confirm the sample for acid fast bacilli at the facility and an aliquot of the sample was then parked in screw cups with double biohazard bags inside a cooler box and transported to Kenya Medical Research Institute (KEMRI) Microbiology reference Laboratory in Kisian for further confirmatory staining, culturing and molecular drug resistance testing. Local specimen shipment was done according to regulation provided by the International Air Transport Association (http://www.iata.org/ ads/issa/htm).

At Kenya Medical Research Institute Microbiology reference laboratory, sputum samples together with the laboratory request form were received from health facilities within the County and checked for completeness in filling the laboratory request form, correct sample tube labelling and leakage. Those meeting the acceptance criteria were given laboratory study number and refrigera-

ted at minus four (- 4°C) awaiting processing.

Sample Decontamination and Microscopy

Decontamination of sputum specimen was done using the *N*-acetyl-l-cysteine-sodium citrate-NaOH (NALC-NaOH) method.[29] Samples were then decanted following centrifugation at 3000 g for 15 min, and the pellets re-suspended to make 3 ml using phosphate buffer solution. Four aliquots of 1.0 ml were made from the stock sample, 1 aliquot was used for florescent microscopy, another for phenotypic DST, Line Probe Assay and the remaining stored at – 80 °C as back up. Staining and microscopy was done as follows; Carbol Fuschin was used to flood heat-fixed sputum sample smears. The slide that was flooded was flame heated, after 10 minutes it was washed with deionised water, decolourised with 3% acid alcohol, flooded with malachite green and left for 2 minutes to stain. This stain was then washed with water and smear air dried and later observed microscopically using X100 oil immersion objective.[30] Microscopy was done to all 256 Sputum samples.

Phenotypic Testing

Phenotypic drug resistance testing for *M. Tuberculosis* was done for first line anti tuberculosis drugs using BACTEC™ MGIT™ 960 system (MGIT) (Becton Dickinson (BD) Bioscience, Erebodegem, Belgium) system in the KEMRI Tuberculosis Microbiology Laboratory. After decantation of sediments to be cultured, a vial of Mycobacteria Growth Indicator Tube (MGIT) containing a lyophilised mixture of antimicrobials was reconstituted with 15.0 ml of Mycobacteria Growth indicator supplement. A micropipette was then used to transfer, 0.8 ml of the mixture to each MGIT tube to be inoculated with specimen including both negative and positive controls.

Using a sterile pipette, 0.5ml of the sample was added to labelled MGIT tubes that were closely tightened and inverted a couple of times to get a uniform constitution. The MGIT tubes were then inserted into the BACTEC machine after scanning each tube.[30] The instrument was maintained at a temperature of 37°C + or - 1°C, which was the optimum growth temperature for *M. tuberculosis*.

Mycobacteria Growth Indicator tubes were incubated until flagged positive by the instrument, negative tubes were flagged negative after a maximum period of 6 weeks when no growth occurred. The positive flags were removed and scanned on the instrument which was followed by visual observation of the tube.

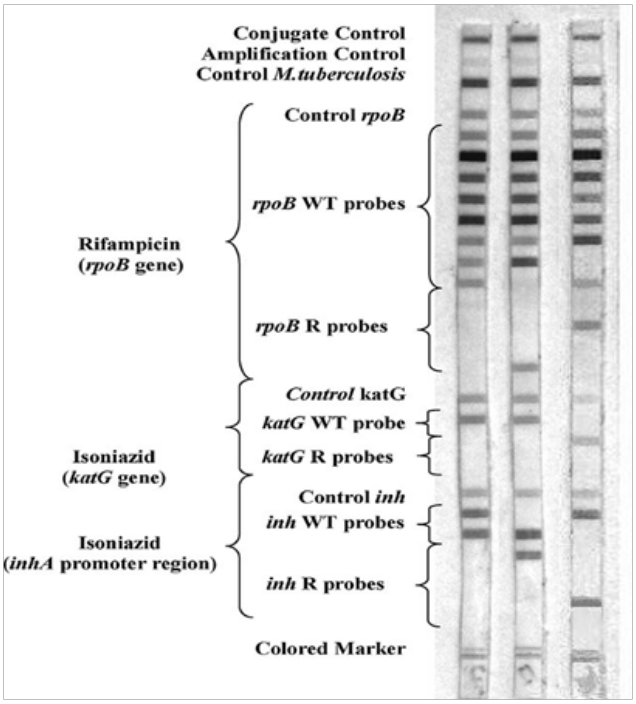
Line Probe Assay

GenoType® MTBDR*plus* assay for detection of first line resistance was performed according to the manufacturer's recommendations (Hain Life Science GmbH, Nehren, Germany). Using multiplex PCR, GenoType® MTBDR*plus* assay was used to target specific mutations in the Rif-resistance determining region (RRD) of the rpoB gene (from codon 505 to 533) to detect rifampicin resistance and mutations in the *inhA* promoter (from -16 to – nucleotides upstream) and *katG* (Codon 315) regions for isoniazid resistance. The genes responsible for first line drug resistance such as *katG*, *inhA*, *rpoB* were amplified and the resulting biotin-labelled amplicons were hybridised to DNA probes bound to membrane probe. For amplification 35µl of a primer nucleotide mixture, buffer for amplification containing 5µl mM MgCl2, 2.5µl of deionised water, 2.5µl Taq polymerase (ROCHE, Mannheim, Germany), and five microlitre of DNA to make a final volume of 50µl. The protocol for amplification consisted of denaturation of 15 min at 95°C then followed by 10 cycles of 30 seconds at 95°C and 2 minutes at 58°C, an additional 20 cycles comprising 25s at 95°C then at 53°C to 70°C of 40 seconds each, and finally extended at 70°C for 8 minutes. Binding of the single stranded amplicons to probes bounded on membrane strips followed by addition of conjugate, then substrate to detect band patterns that are visible on the strip. Then strips were allowed to dry and interpreted according to the instructions provided by the manufacturer. For each gene, GenoType MTBDRplus assay detects the presence of mutant and wild type probes.

Interpretation

Each strip of Line Probe assay had 27 reaction zones and these included 6 controls bands, namely; conjugate band, M. tuberculosis complex, amplification, *rpoB*, inhA and katG, 8 *rpoB* wild type (*WT1–WT8*) and 4 mutant probes (*rpoB MUT D516V, rpoB MUT H526Y, rpoB MUTS531 L and rpoB MUT H526D*), one katG wild type, 2 mutant and 2 *inhA* wild type and 4 mutant probes and *inhA MUT3B T8A* (Figure 1).

FIGURE 1. GenoType MTBDRplus Bands



Source: GenoType MTBDRplus test (Hain Life science GmbH, Nehren, Germany

Either missing wild type band or the presence of mutant band was taken as a symbol of a resistant strain. To prov-

ide a consistent result, all the 6 expected control bands had to appear correctly, otherwise, the result was considered invalid.

Data Management
Data Collection and Storage
The study employed questionnaires, clinical reports and laboratory test reports as the tools for collecting data. Study Participants who met the inclusion criteria were explained to the purpose of the study, possible risk and benefits and those who agreed to participate in the study were duly informed, consented and enrolled into the study.

This study had both paper and electronic study forms for each patient, linked by a unique study Identification (ID) number given to each participant. The list linking study ID numbers with specific individuals (face sheet) was stored separately and securely, i.e., in a different physical location, in a locked Good Clinical Practice (GCP) compliant cabinet at Kenya Medical Research Institute, Centre for Global Health(KEMRI-CGHR)-Kisumu.

The first form contained all demographic data and clinical data for the participants. The second form was a laboratory request form and contained all laboratory test results such as mycobacteriology data, smear microscopy, DST and LPA test results and dates of all tests. All study form data were kept in locked Good Clinical Practice complaint file cabinets in the clinics and laboratories at KEMRI-CGHR -Kisumu. Electronic files in the electronic database were stored in the password-protected computers. Confidentiality was assured by ensuring that all data-containing study forms and specimens were identified using study ID numbers unique to each participant.

All laboratory information was communicated directly to the clinicians and collated in the patients' charts and on the laboratory study forms. Only clinical and study staff had access to information collected or generated as part of this study.

Data Analysis
Data was collected onto paper and electronic forms and then entered into Laboratory Information management system database. The study database had quality check codes built in and was also checked against primary sources from clinicians or laboratory technicians. Monitoring of the study site was conducted every month. Statistical Package for the Social Sciences (SPSS) v23 (SPSS Software | IBM) was used for data analysis and it merged the clinical and the laboratory databases prior to analysis.

Demographic data such as Sex, Age were analysed using Descriptive Analysis. Mean was used to determine the mean Age among sample Tuberculosis cases while mode was used to determine the modal sex mostly affected by Tuberculosis drug resistance. Frequency tables and bar charts were used to present this data. Inferential statistics was used to analyse categorical test results such as New and previous TB cases, HIV status, bacteriological smear and Culture MGIT Test results. Multiple response LPA drug resistance results had the variables defined and presented in frequency tables. Chi square test was used to assess the association between HIV status and drug resistance conferring mutations in Kisumu County.

Cross tabulation was used to explore First line drug resistance mutation patterns among new and previously treated cases and factors associated with drug resistance. Associations were considered statistically significant when *p-value* was less than or equal to *.05*.

Ethical Considerations
Linking of identifying information such as patient name and birth date to the study identification number appeared only on a cover sheet of the patient's data form. These data forms were kept in a locked GCP-compliant cabinet at Kenya Medical Research Institute, Centre for Global Health Research, Kisumu and were only accessible by the investigation team. Sputum samples were labelled with the date of collection and patient's study identification number. After the end of the study, the cover sheet was destroyed, unlinking the study identification number and de-identifying the data. Data entry was performed on site by the local investigators on a password protected computers and only the study investigators and data staff had access to this data.

Direct LPA which is not a routine practice was used to shorten laboratory turnaround time. Ethical approval was provided by Kenya Medical Research Institute, Scientific Ethical Review Unit (KEMRI/SERU/CGHR/002-02-330/4079) and National Commission for Science, Technology & Innovation (NACOSTI/P/21/10981).

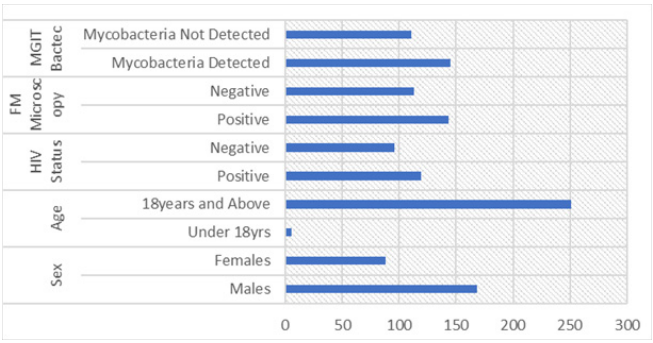
RESULTS
General Characteristics of the Study Population
A total of 256 sputum samples from Tuberculosis clinical suspected cases from Kisumu County, Kenya for period of 12 months, November 2020 to October 2021 were included in the study. The samples received were classified as new TB cases and previously treated cases as per the WHO guidelines for surveillance of drug resistance in tuberculosis.

Socio-Demographic Characteristics of the Respondents
Out of 256 study participants, 216 had their HIV status known while 40 did not respond (none response) to the HIV status question. Of the 216 respondents with known HIV status, 37 (17.1%) were new cases, while 179 (82.9%) were retreatment cases. Out of the 216, 137(63.4%) were male while the remaining 79 (36.6%) were female. Aged below 18 years were 5(2.3%), while aged 18 years and above were 211(97.7%). The ages showed a normal distribution with a mean age of 40 years, a Standard Deviation of ± 12.9 and a range of 13 to 77 years. Data on drug resistance stratified by age group and sex provides insight into risk groups and effectiveness of specific TB control activities. Additionally, the magnitude of drug resistance among younger age group is more likely to be indicative of recent transmissions than among older age group, who may be harbouring older infections. From the sample, HIV positive cases were 119(55.1%) and negative were 97 (44.9%). None response for HIV status were 41 participants. (Figure 2)

Characteristic of TB Confirmed Cases
Out of a total of 145 Mycobacteria confirmed cases on MGIT BACTEC from Tuberculosis suspected cases, 32 (22

%) were from new TB cases and 113(78%) from retreatment cases. Males were 112 (77.2%) while females were 33(22.8%) for bacilli confirmed cases. Aged below 18 years were 5(2.3%), while aged 18 years and above were 211(97.7%), 119(55.1%) were positive while 97 (44.9%) were negative. (Figure 3).

FIGURE 2. Socio-Demographic Characteristics of the Respondents



Factors Associated with HIV Status Outcome

Out of 37 Tuberculosis new cases, 15(40.5%) were positive for HIV while positivity among on treatment cases was 104(58.1%). Chi square test for TB cases and HIV Status showed (χ2=3.822, df=1, *P=.051*), (OR=0.49,95%CI (0.23-1.01). Out of 121 samples that showed mycobacteria detected from BACTEC Culture, 75(62%) were positive for HIV while 46(38%) were HIV negative. Chi square test for BACTEC culture results and HIV status outcome was (χ2=5.28, df=1, p=0.022), (OR=1.89,95%CI:(1.09-3.20).

Out of 84 samples that were positive on FM AFB, 46(54.8%) were positive for HIV, while 38(45.2%) were HIV negative. Chi square test for FM AFB and HIV status was (χ2=0.006, df=1, p=0.938), (OR=0.98, 95%CI: (0.71-1.38). Out of 87 samples that showed mycobacteria detected from First line LPA, 53(60.9%) were positive for HIV while 34(39.1%) were negative. Chi- square test for FL LPA and HIV Status was (χ2=1.99, df=1, *p=.157*), (OR=1.27, 95%CI: (0.91-1.78). (Table 1)

Phenotypic and Molecular Drug Resistance among HIV Cases

First line phenotypic drug resistance for Isoniazid showed a total of 11(5.1%) out of which 8 (6.7%) were HIV positive and 3(3.2%) were HIV negative cases. Chi-square test of association between FL DST for isoniazid resistance and HIV status showed that (χ2=1.457, df=1, *p=.186*), (OR=2.17,95%CI :0.59-7.97)).

First line phenotypic rifampicin resistance showed that 10(4.6%) were resistance detected, out of which 8 (6.7%) were HIV positive and 2(2.1%) HIV negative. Chi- square test of association between rifampicin resistance and HIV status showed (χ2=2.62, df=1, *p=.095*), (OR=3.3,95%CI (0.71-14.9). First line LPA drug resistance for isoniazid showed that out of 9(4.2%) that were resistance detected, 6(5.0%) were HIV positive while 3(3.1%) were HIV negative. Chi- square test of association between FL LPA drug resistance for isoniazid and HIV Status showed (χ2=0.508, df=1, *p=.36*), (OR=1.63,95%CI :0.42-6.35). First line LPA drug resistance for rifampicin showed that out of 10(4.6%) that were resistance detected, 8 (6.7%) were HIV positive while 2(2.1%) were HIV negative. Chi-square test of association between FL LPA drug resistance for rifampicin and HIV status showed (Chi-Square=2.742, df=1, *p=.36*), (OR=4.89,95%CI: 0.59-39.94). (Table 2).

Mutant and Wild Type Gene Probes

The study found out that mutant probes among the HIV positive were *inhA MUT1* 1(0.7%), katG *MUT1* 4(2.6%), *roB MUT2A* 3(2.1%), *roB MUT3* 1(0.7%), *roB MUT3/katG MUT1* 1(0. 7%). Mutant probes among the HIV negative were *inhA MUT1* 1(0.7%), *katG MUT1* 1(0.7%) and *roB MUT2A* 1(0.7%). Wild Type gene deletion among the HIV positive cases were observed in probes *katG WT* 3(2.1%), *roB WT7*, *katG WT* 1(0.7%). Wild Type gene deletion among the HIV negative cases were *inhA WT1* 1(0.7%), *inhA WT1/inhAWT2* 1(0.7%), *katG WT* 1(0.7%). (Figure 4)

Codon and Amino Acid Change among HIV Outcome

Codons analysed among the HIV positive participants were; codon-*15* 1(0.7), codon *315* 4(2.8%), codon *526* to *529* 4(2.8%), codon *530* to *533* 2(0.9%). Codons analysed among the HIV negative participants were; codon-*15* 2(1.4%), codon *315* 1(0.7%), codon *526* to *529* 1(0.7%). Amino acid changes among the HIV positive cases were; *C15T* 1(0.7%), *H526R*, *S315T1* 1(0.7%), *H526Y* 3(2%), *S315T1* 3(2%), *S531L* 1(0.7%), *S531L*, *S315T1* 1(0. 7%). Among the HIV negative cases were; *C15T* 2(0.9%), *H526Y* 1(0.5%), *S315T1* 1(0.5%). Out of 9 (4.2%) that were INH resistance on Line Probe assay, 6(5.0%) were from HIV positive cases while 3(3.1%) were from HIV negative cases. Additionally, out of 10(4.6%) that were rifampicin resistance, 8(6.7%) were from HIV positive cases while 2(2.1%) from HIV negative cases. All the 2 MDR cases arose from positive participants representing (0.02%) of the total HIV positive participants. (Figure 5)

DISCUSSIONS

Out of 256 study participants, 216 had their HIV status known while 40 did not respond (none response) to the HIV status question. This study found out that out from the response of 216, there were more males 137(63.4%) compared to females 79 (36.6%). This is in agreement with the WHO report that indicate that relatively more males than females are exposed to Tuberculosis and this could be attributed to the difference between the two sex groups in biological, societal role and access to health facilities.[8] Majority of participants were aged 18 years and above 211(97.7%) while the remaining 5(2.3%) were aged under 18 years.

All the patients had a mean age of 40 years with a Standard Deviation of ± 12.9 and a range of 13 to 77 years. The current study is in good agreement with a study reporting that the 31 to 40 years' age group was the most predominant group for isolation of DR-TB and that male population was at the highest risk.[31] According to Ahmed et al in a study that was conducted in India which is one of the high burden Tuberculosis countries, it was found that 17.2% of collected samples were from

TABLE 1: Factors Associated with HIV Status Outcome

		No of Patient (%) n=216	HIV Positive	HIV Negative	P-Value	OR (95%CI)
TB Case	Retreatment	179(82.9)	104(58.1)	75(41.9)	0.51	1.40(1.00-19)
	New TB Case	37(17.1)	15(40.4)	22(59.5)		
MGIT_ BACTEC	Mycobacteria Detected	121(56)	75(62)	6(47.4)	0.022	1.89(1.09-3.20)
	Mycobacteria Not Detected	95(44)	44(46.3)	51(52.6)		
FM_AFB	Positive	119(55.1)	46(38.7)	38(39.2)	0.938	0.98(0.71-1.38)
	Negative	97(44.9)	73(61.3)	59(60.8)		
LPA Tuberculosis	Mycobacteria Detected	87(40.3)	53(44.5)	34(35.1)	0.157	1.27(0.91-1.78)
	Mycobacteria Not Detected	129(59.7)	66(55.5)	63(64.9)		

TABLE 2: Phenotypic and Molecular Drug Resistance among HIV Cases

	HIV Positive N(%)	HIV Negative N(%)	Total Resistance N(%)	P Value	OR (95%CI)
DST Isoniazid	8(6.7)	3(3.2)	11(5.1)	.227	2.17(0.59-7.97)
DST Rifampicin	8(6.7)	2(2.1)	10(4.6)	.105	3.3(0.71-14.9)
LPA Isoniazid	6(5.0)	3(3.1)	9(4.2)	.476	1.63(0.42-6.35)
LPA Rifampicin	8(6.7)	2(2.1)	10(4.6)	.98	4.89(0.59-39.94)

FIGURE 3. Characteristic of TB Confirmed Cases

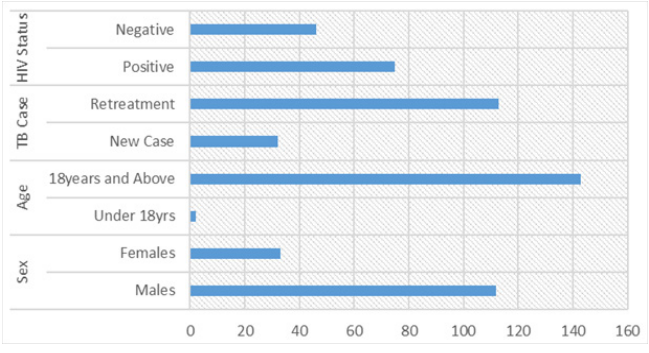
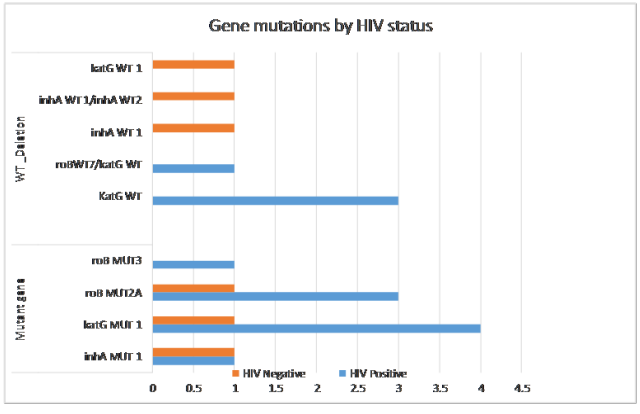


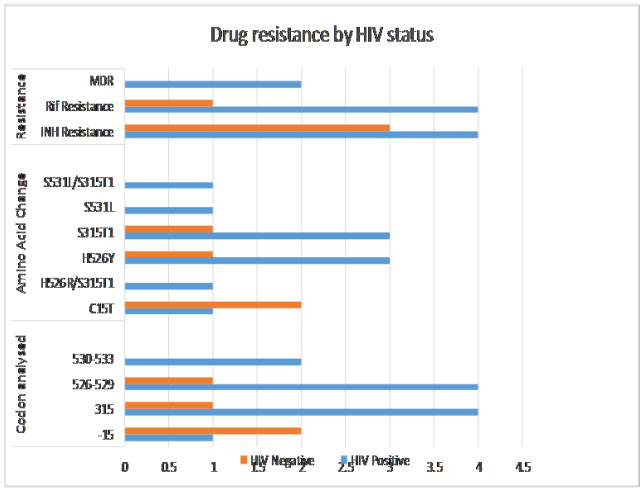
FIGURE 4. Chart of Mutant and Wild Type Gene Probes



new cases and 82.8% were from previously treated cases.[32] These findings are consistent with the current study that found out that majority of TB cases were retreatment cases 179(82.9%) while new Tuberculosis cases were 37 (17.1%). Children under the age of 18 years were more likely to be associated with isoniazid resistance (OR 5.02,95%CI:0.785-32.095) and resistance to rifampicin (OR 5.58,95%CI:0.862-36.072) compared to adults of 18 years and above. Additionally, the study showed that females were more likely to develop isoniazid resistance, Odds Ratio (OR 1.59,95%CI:0.499-5.067) and rifampicin resistance (OR 2.86,95%CI:0.83-9.882) compared to males.

General isoniazid resistance was 11(5.1%) in all the cases while rifampicin was 10(4.6%) across all the TB cases.

FIGURE 5. Chart of Mutant and Wild Type Gene Probes



This study showed that, out of a total 145 that confirmed mycobacteria detected, there was anon response of 24 for the variable HIV status. Out of a total of 121 confirmed Tuberculosis cases that responded to the HIV status variable, 75(62.0%) were HIV positive while 46(38.0%) were HIV negative. Positivity among the new cases was 15(40.4%) while positivity among the on-treatment cases was 104 (58.1%). According to a study conducted among drug naïve patients in Nairobi Kenya, HIV increased the incidence of TB and the risk of TB infection by 16 to 27 times in PLHIV than in the general population.[12]

Sing et al also estimated that PLHIV, especially with fewer than 200/cm3 CD4 count show a 19 (15 to 22) -fold increased risk of developing active TB compared with those who are HIV negative.[5] These findings are consistent with the current study that found out that HIV status and Tuberculosis cases were significantly associated at $p<.05$. Specifically, HIV positive cases were more likely associated with retreatment cases (OR 2.0,95%CI:1.0-4.3) compared to new cases. First line Phenotypic drug resistance for isoniazid showed a total of 11(5.1%) out of which 8 (6.7%) were HIV positive and 3(3.2%) HIV negative, while rifampicin resistance showed that 10(4.6%) were resistance detected, out of which 8 (6.7%) were HIV positive and 2(2.1%) HIV negative. First line LPA showed that out of 9 (4.2%) that were INH resistance, 6(5.0%) were from HIV positive cases while 3(3.1%) were from HIV negative cases.

Additionally, out of 10(4.6%) that were rifampicin resistance, 8(6.7%) were from HIV positive cases while 2(2.1%) from HIV negative cases. Of all the MDR cases, 2 were from HIV positive participants which was an indicator of poor treatment outcome for HIV cases.

The Odds ratio for rifampicin resistance among the HIV positive cases was higher for both DST rifampicin (OR 3.3,95%CI:0.71-14.9) and LPA rifampicin (OR 4.89,95%CI:0.59-39.94) compared to isoniazid DST isoniazid (OR 2.17,95% CI:0.59-7.97) and LPA isoniazid (OR 1.63,95%CI:0.42-6.35).

Chi square test showed that there was no significant relationship between Tuberculosis drug resistance and HIV for phenotypic Isoniazid resistance, ($\chi2=1.457$, df=1, $P=.227$) and rifampicin resistance, ($\chi2=2.629$, df=1, $P=.105$) and molecular Isoniazid resistance ($\chi2=0.508$, df=1, $P=.476$) and rifampicin resistance ($\chi2=2.742$, df=1, $P=.98$). These findings are consistent with findings from Khan et al that showed that there was no evidence that HIV infection modifies the fitness of drug-resistant strains.[26,33,34]

The study found out that HIV positive clients had high INH mutations in the promoter region of inhA gene at codon *-15* with amino acid change of *S315T1*, while low INH resistant strains had mutations in the *katG* gene at codons *315*. Additionally, HIV positive clients experienced mutations at codons *526* to *529* and *530* to *533* in the *rpoB* genes with amino acid changes *H526Y* and *S531L*. All the MDR strains were from HIV positive cases and had mutations in the *rpoB* and *katG* genes. This is consistent with a systematic review from Sultana et al, that found out that the odds of developing MDR-TB in HIV infected patients was 42% higher than those of HIV negative individuals.[35] In other studies conducted in regions of HIV prevalence, there was growing evidence to suggest that infection with more than one strain occurred.[36,37] The r*poB* gene displayed mutations at codons *530* to *533* with amino acid changes of *S531L* and *S315T1*, while *katG* had mutations at codon *526* to *529* and *315* with amino acid changes of *H526R* and *S315T1*. HIV negative clients experienced mutations in the inhA, katG and rpoB genes.

The study found out that the frequent mutant probes among the HIV positive was *katG MUT1* 4(2.8%), while common mutant probes among the HIV negative was *katG MUT1* 1(0.7%) and *roB MUT2A* 1(0.7%). Wild Type gene deletion among the HIV positive cases were observed highest at probes *katG WT* 3, whereas Wild Type gene deletion among the HIV negative cases were associated mostly with h probes *inhA WT1* 1(0.7%), *katG WT* 1(0.7%). Greater variability and unknown mutations was observed in mutant probes from HIV positive cases than in HIV negative cases.

CONCLUSION

This study showed that there was no significant relationship between Tuberculosis drug resistance and HIV status. This could be attributed to lack of comprehensive data stratification on HIV testing and TB drug resistance testing. Surveillance data from different studies in different geographical settings have attributed discordant associations due to heterogeneity in setting, demographic profile, methodology and analysis of data. Children under the age of 18 were more associated with isoniazid and rifampicin resistance compared to adults. The magnitude of drug resistance among younger age groups is more likely to be indicative of recent transmission from older age groups, who may be harbouring older infections. The County and National government should strengthen Tuberculosis drug resistance surveillance among children especially in HIV high burden regions like Western Kenya. Additionally, a greater variability in mutations and presence of unknown mutations were observed in HIV positive participants compared to HIV negative patie-

nts. This could be an indicator of poor outcomes for Tuberculosis patients who are co-infected with HIV. Understanding Tuberculosis molecular epidemiology and its variability among the HIV prevalent populations emphasizes the need for research in HIV-endemic settings to develop appropriate regional specific interventions for drug resistant tuberculosis. Greater changes in amino acid sequences among retreatment cases compared to new cases that were observed may be an indication that such mutations might be acquired during treatment courses by repeated administration of the same anti-TB drugs. Further population-based studies are required to guide policies on transmission of drug-resistant tuberculosis strains in HIV endemic settings like Kisumu County, Western Kenya.

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Peer Reviewed

Competing Interests: None declared.

Funding: This study was not funded

Received: 14 October 2021; **Accepted:** 26 October 2021

Cite this article as Ogumbo F, Odero R, Odhiambo B, Emojong P, Okumu A, Nonoh J, Wandiga S, Guya B. Isoniazid and Rifampicin Tuberculosis Drug Resistance in HIV Endemic Region of Western Kenya. *East Afr Sci J.* 2022;6(1):37-47. https://doi.org/10.24248/easci.v4i1.57

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