Mutations in *Mycobacterium tuberculosis* that Confers Resistance to First Line and Second Line Anti Tuberculosis agents in Western Kenya

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Abstract

BACKGROUND

Tuberculosis drug resistance remains a global, regional as well as a national public health concern, and is driven by point mutations in the *Mycobacterium tuberculosis* genome. However, their frequencies vary geographically and that affects the general applicability of antimicrobial agents as opposed to regional tailored or individualized chemotherapy.

MATERIALS AND METHODS

In this prospective cross-sectional study, sputum samples were collected from 256 tuberculosis clinical suspects attending various health facilities in Kisumu County between November 2020 and October 2021. The study aimed to describe the Mutations in *Mycobacterium tuberculosis* that confers resistance to first and second-line anti-tuberculosis agents in Kisumu County, Western Kenya. Detection of mutations conferring resistance to anti-tuberculosis drugs was carried out using GenoType MTBDR*plus* and GenoType MTBDR*sl*.

RESULTS

Out of a sample of 256 Tuberculosis suspect cases, 145 were *Mycobacterium tuberculosis* bacilli confirmed, out of which 32 (22%) were from new TB cases and 113(78%) retreatment. High isoniazid-resistant strains had mutations in the promoter region of *the inhA* gene at codon -15 with an amino acid change of *S315T1*, while low isoniazid-resistant strains had mutations in the *katG* gene at codon *315*. Among rifampicin-resistant strains, four isolates displayed mutations at codon *526* to *529* in the *rpoB* gene with an amino acid change of *H526Y* and one isolate displayed mutation at codon *530* to *533* in the *rpoB* gene with an amino acid change of *S531L*. The MDR strains had mutations in the *rpoB* and *katG* genes. Additionally, greater variability of mutations was exhibited among retreatment and HIV-positive cases. No drug resistance-conferring mutations were detected against second-line anti-tuberculosis drugs.

CONCLUSION

A greater variability of mutations was observed from isoniazid and rifampicin resistance in retreatment cases compared to new cases and additional mutations were more associated with HIV positive cases compared to HIV negative cases.

Keywords: *Mycobacterium tuberculosis, genetic mutations, drug resistance, Kenya*

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Introduction

Globally, the prevalence of drug-resistant tuberculosis (DR-TB) has increased substantially in the past 20 years making Tuberculosis (TB) the leading cause of death from a single infectious disease agent and the leading cause of death among persons living with human immunodeficiency virus (HIV) infection, accounting for approximately 40% of deaths in 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 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 (4,5). Rifampicin-resistant (RR) or multidrug-resistant (MDR) TB occurred among 3.6% and 18% of new and previously treated TB cases, respectively (5.6% among all cases) (6). Spontaneous chromosomal point mutations are the main mechanism underlying drug resistance in TB (7) and a limited number of mutations account for a majority of the phenotypic resistance to first- and second-line anti-TB drugs. Resistance to Fluoroquinolones(FQ), the most effective second-line anti-TB drugs used to treat MDR-TB, is associated with mutations in a short discrete region of the *gyrA* gene and less frequently *gyrB,* commonly referred to as the quinolone-resistance determining region (QRDR) (8). Kenya is among the 14 countries globally that are in all three lists of high burden countries for TB, TB/HIV and MDR-TB and the fifth highest burden in Africa (9). The estimated incidence of TB in the country is 348/100,000 population, translating to about 169,000 TB cases occurring annually and the mortality rate (excluding HIV+TB) is 60/100,000 population (10). According to WHO, in 2018 the MDR-TB prevalence in Kenya was 1.3 % in new cases and 4.4 % in retreatment cases (11). Tuberculosis affects all age groups but had its 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 (12). A study done on the prevalence and detection of drug-resistant mutations in *Mycobacterium tuberculosis* among drug naïve patients in Nairobi Kenya from 2015 to 2017 found that out of 132 patients that were tested for drug resistance, two patients showed resistance associated with first and second-line TB drugs (13). Of these two patients that had resistance, one showed resistance to Isoniazid (INH), while the other indicated a case of MDR, showing resistance to both INH and rifampicin (RIF) (13). Out of the 132 patients tested for resistance to second-line anti -TB drugs, one cross-resistance was detected for aminoglycosides and fluoroquinolones (13). In Western Kenya, anti-tuberculosis drug resistance is an emerging health concern, especially in Kisumu County where cases of HIV and TB co-infection are predominant (14). 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 (13). According to reports from Kenya National Tuberculosis, Leprosy and Lung Disease Program, Kisumu County had the third highest TB co-infection rate in Kenya at 59%, Homabay at 64% and Siaya at 63% which was way above the national co-infection of 28% (12). According Ministry of Health report on Kenya's HIV Estimates, Kisumu County has the second highest HIV prevalence of 18.6% after Homabay 20.2% and Siaya 17.8% against the national prevalence of 4.5% (12). This high prevalence possess a greater challenge in tuberculosis control and drug resistance in Kisumu County as HIV is more likely associated with TB (11). GenoType MTBDR*plus* and GenoType MTBDR*sl* assays are molecular methods that have been approved by WHO for

the detection of mutations conferring resistance to anti-tuberculosis drugs (15). To monitor drug resistance molecularly, the magnitude of drug resistance-conferring mutations in a specific geographical setting needs to be identified, and such information is presently missing for Tuberculosis patients in Kisumu County, Western Kenya. To bridge this knowledge gap, this study aimed to describe Mutations in *Mycobacterium tuberculosis* that confers resistance to first and second-line anti-tuberculosis drugs in Kisumu County, Western Kenya.

Materials and methods

Study site

This study was carried out in Kisumu County, Western Kenya. The County has a total population of 1,153,343. The TB prevalence rate in Kisumu county 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% (16).

Study design

This was a hospital and laboratory-based descriptive cross-sectional study design. We collected data from Tuberculosis patients attending TB clinics and hospital facilities within Kisumu County. This study was conducted between November 2020 and October 2021 to describe the profile of drug resistance-conferring mutations among new and previously treated pulmonary tuberculosis cases from Kisumu County, Kenya.

Inclusion criteria

Informed consent (or parental permission), after demonstrating understanding formed the basis for recruitment. The patients enrolled in the study had to be clinically presenting as a TB case as per the Ministry of Health's case definition for suspected tuberculosis cases in Kenya.

Sampling technique

This study employed 100 per cent sampling of all clinically suspected Tuberculosis patients attending various health facilities in Kisumu County. Saturated sampling was preferred in this study because TB Clinics and Hospital facilities within Kisumu County were too few. All health facilities in the county were used as recruitment centres.

Data collection

The study employed clinical case reports and laboratory test reports as tools for collecting data. Study participants who met the inclusion criteria and consented to participate in the study were enrolled in the study.

Sample collection

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 bacteriology 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, Kisumu county for further confirmatory staining, culturing and Molecular drug resistance testing. Local specimen shipment was done according to regulations provided by the International Air Transport Association (http://www.iata.org/ ads/issa/htm).

Phenotypic testing

Phenotypic drug resistance testing of *Mycobacterium tuberculosis* isolates to the first and second-line drugs was done using BD BACTEC MGIT 960 system in the KEMRI Tuberculosis Microbiology Laboratory. After decantation of sediments to be cultured, a vial of mycobacteria growth indicator tube (MGIT)

containing a lyophilized mixture of antimicrobials was reconstituted with a 15.0 ml MGIT growth supplement provided. A micropipette was then used to transfer, 0.8 ml of the mixture to each MGIT tube to be inoculated with specimens including both negative and positive controls. Using a sterile pipette, 0.5 ml of the well-mixed processed sample was then added to the corresponding labelled MGIT tubes. The tubes were closed tightly and inverted three times to allow the proper constitution of the mixture. The MGIT tubes were then inserted into the BACTEC machine after scanning each tube (17). The instrument maintained a temperature of 37 °C + or - 1 °C, which was the optimum growth temperature for M. tuberculosis. MGIT tubes were then incubated until the instrument flagged them positive, as for the negative tubes, they were flagged after a maximum of 6 weeks when no growth occurred.

Line probe assay

Using multiplex Polymerase Chain Reaction, GenoType MTBDR*plus* and GenoType MTBDR*sl* assays were used to target specific mutations that confer resistance to first and second-line anti-tuberculosis drugs respectively. GenoType MTBD*plus* was used to detect mutations in the Rif-resistance determining region(RRD) of the *rpoB* gene to detect rifampicin resistance and mutations in the *inhA* promoter and *katG* regions for isoniazid resistance. The genes *katG*, *inhA, and rpoB* are responsible for first-line drugs.

GenoType MTBDR*sl* was used to target mutations in the quinolone-resistance determining region (QRDR) of *gyrA* and *gyrB* genes for detection of resistance to fluoroquinolones and the *rrs* and *eis* promoter region for detection of resistance to second-line injectable drugs. For amplification to occur, 35µl of a primer and nucleotide mixture, buffer containing 5µl mM MgCl2, 2.5µl deionised water and 2.5µl Taq DNA polymerase (ROCHE, Mannheim, Germany), and 5 µl of DNA to a final volume of 50µl was used. The amplification process consisted of denaturation, annealing, elongation and a final extension at 70°C for 8min. Hybridization of the single-stranded amplicons to membrane-bound probes on the strip followed by addition of conjugate, and substrate to detect visible band patterns on the strips.

Data management and analysis

SPSS version 23 was used for data analysis and it merged the clinical and the laboratory databases before analysis.

Descriptive statistics were used to analyze Demographic data such as Age, Sex. Descriptive methods such as measures of central tendency (mean and mode) and measures dispersion (standard deviation, variance, range) were used. Frequency tables and bar charts were used to present this data. Inferential statistics were used to analyze categorical test results such as New and previous TB cases, HIV status, and Culture MGIT Test results. Multiple response Line Probe Assay (LPA) drug resistance results had the variables defined and presented in frequency tables. Cross tabulation was used to explore First line and second-line drug resistance mutation patterns among new and previously treated cases and second-line drug resistance mutations in new and previously treated TB cases. Chi-squared test was applied to assess factors associated with drug resistance TB in terms of the odds ratio and its 95% confidence interval (CI).

Differences were considered significant when the p-value was less than or equal to 0.05.

Ethical considerations

Ethical approval was obtained from the Kenya Medical Research Institute (KEMRI), Scientific Ethical Review Unit (SERU) (KEMRI/SERU/CGHR/002-02-330/4079) and National Commission for Science, Technology & Innovation. This study was conducted according to the requirements of the Helsinki declaration.

Results

Sub-county TB case profile

A total of 256 sputum samples from tuberculosis clinical suspected cases from Kisumu County, Kenya for a period of 12 months, November 2020 to October 2021 were included in the study. Out of the sample size, 145 (56.6%) confirmed *Mycobacterium tuberculosis* cases while 111 (43.4%) were negative for *M. tuberculosis*.

Among the confirmed cases, Kisumu West Sub-county had the highest number of cases 50 (34.5%), while Kisumu East and Nyakach Sub-Counties had 1 (0.7%) each. **(Figure 1)**

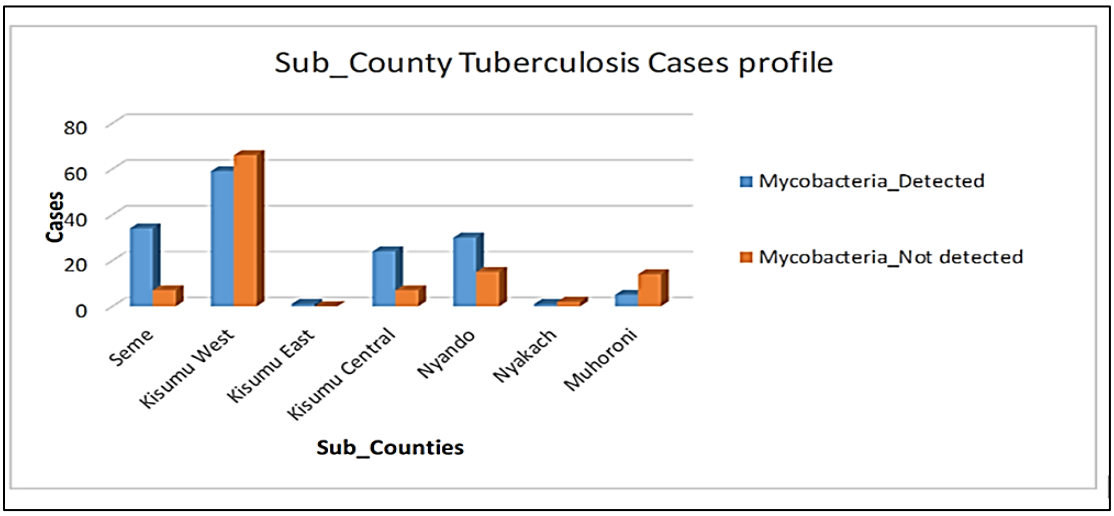

Figure 1:
Sub-county tuberculosis cases profile

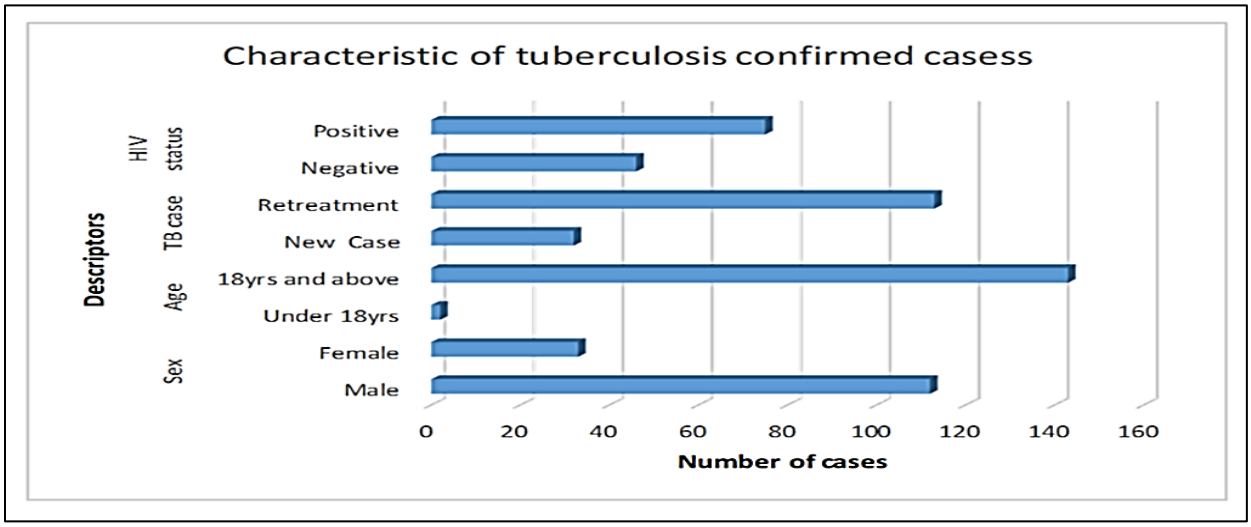

Figure 2:
Characteristics of TB Confirmed cases

Characteristics of TB cases

Out of a total of 145 *Mycobacterium tuberculosis* confirmed cases on MGIT BACTEC from tuberculosis suspect cases 32 (22.1%) were from new TB cases and 113 (77.9%) retreatment. Males were 112 (77.2%) while females were 33 (22.8%). Ages under 18 years were 2 (1.4%), while 18 years and above 143 (98.6%), while 75 (51.7%) positive while 46 (31.7%) negative. None response for this variable was 24 (16.6%). **(Figure 2).**

Molecular drug resistance among HIV cases

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 ($\chi 2$=0.508, df=1, p=0.36), (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=0.36), (OR=4.89,95%CI: 0.59-39.94). **(Table 1).**

Table 1:
Molecular drug resistance among HIV cases

	HIV Positive N (%)	HIV Negative N (%)	Total Resistance N (%)	P Value [95%CI]	OR [95%CI]
LPA Isoniazid	6(5.0)	3(3.1)	9(4.2)	0.476	1.63(0.42-6.35)
LPA Rifampicin	8(6.7)	2(2.1)	10(4.6)	0.98	4.89(0.59-39.94)

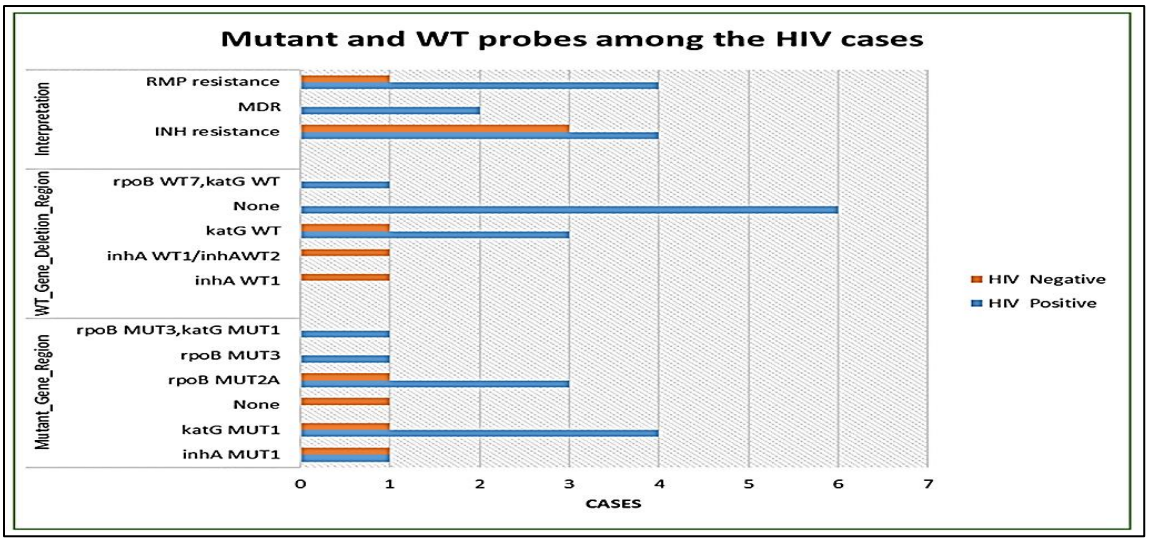

Figure 3:
Mutant and Wild Type gene probes among HIV Cases

Mutant and wild-type gene probes among HIV cases

Of the total of 119 (55.1%) of the HIV positive cases, the study found out that mutant probes among the HIV positive were *inhA MUT1* 1 (0.8%), *katG MUT1* 4 (3.4%), *rpoB MUT2A* 3 (2.5%), *rpoB MUT3* 1 (0.8%), *rpoB MUT3/katG MUT1*1(0.8%). From a total of 97 (44.9%), mutant probes among the HIV negative were *inhA MUT1* 1 (0.5%), *katG MUT1* 1 (1.0%) and *rpoB MUT2A* 1 (1.0%). Wild-type gene deletion among the HIV-positive cases was observed in probes *katG WT* 3 (2.5%), *rpoB WT7, and katG WT* 1 (0.8%). Wild Type gene deletion among the HIV negative cases were *inhA WT1* 1 (1.0%), *inhA WT1/inhAWT2* 1 (1.0%), *katG WT* 1 (1.0%). **(Figure 3).**

Codon and amino acid change among HIV Cases

Codons analysed among the HIV positives were, codon-*15* 1(0.8), codon *315* 4 (3.4%), codon *526 to 529* 4(3.4%), codon *530 to 533* 2(1.7%). Codons analysed among the HIV negative were codon-*15* 2(2.1%), codon *315* 1(1.0%), codon *526 to 529* 1 (1.0%). Amino acid changes among the HIV positive cases were *C15T* 1(0.8%), *H526R, S315T1* 1(0.8%), *H526Y* 3(2.5%), *S315T1* 3(2.5%), *S531L* 1(0.8%), *S531L, S315T1* 1(0. 8%). Among the HIV negative cases, *C15T* 2 (2.0%), *H526Y* 1(1.0%), and *S315T1* 1(1.0%).

Out of seven (3.2%) that were total INH resistance for molecular drug resistance testing, 4 (3.36%) were from HIV positive cases while 3(3.1%) were from HIV negative cases. Additionally, out of 5 (2.3%) that were rifampicin-resistant, 4 (3.4%) were from HIV-positive cases while 1 (1.0%) were from HIV-negative cases. The two (0.9%) MDR cases were from HIV-positive participants. **(Figure 4).**

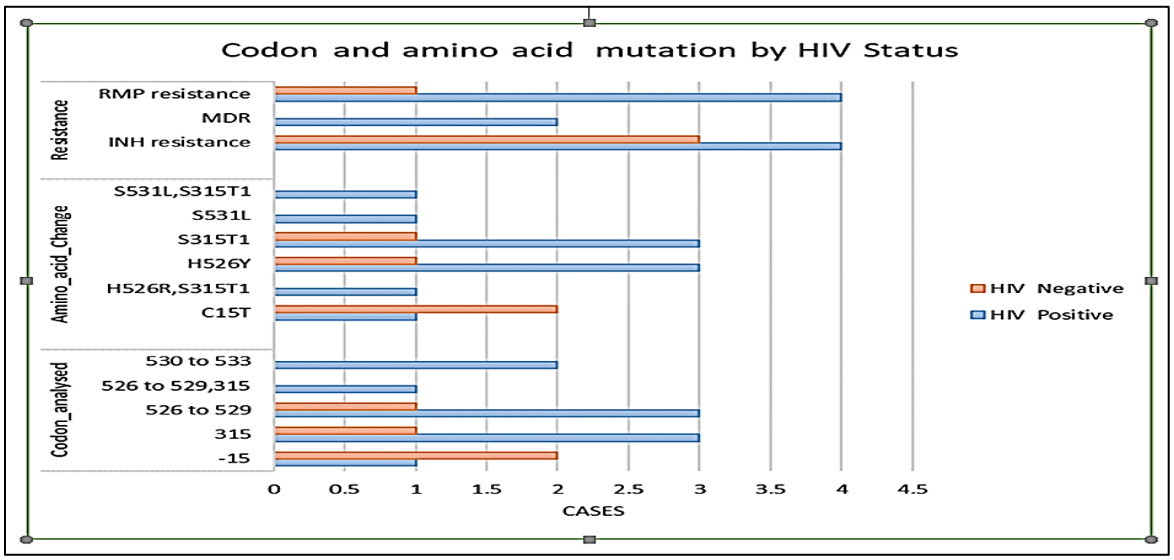

Figure 4:
Codon and amino acid change in HIV status

First-line drug resistance-conferring mutations

Genotype MTBDR*plus*, 7 (4.8%) showed Isoniazid monoresistance, 5 (3.4%) rifampicin monoresistance, while molecular MDR was 2 (1. 4%). GenoType MTBDR*plus* showed that out of the Seven isoniazid resistance, 5 (3.4%) had mutations in the *katG MUT1* showing a high level of isoniazid resistance, while 2 (1.4%) *inhA MUT1* showed a low level of isoniazid resistance. Mutations associated with rifampicin resistance were detected at probes *rpoB MUT2A* 4 (2.8%) and *rpoB MUT3* 1 (0. 7%). Molecular MDR sowed hereto resistance to isoniazid and rifampicin, 1 sample showed mutations in the *rpoB MUT3, katG MUT1* probes while the other deletions in the *rpoB WT7, katG WT*. Two MDR cases were from the same health Centre in the Seme sub-county and had the same mutant gene region and the same amino acid change *S315T1*. There were wild-type gene deletions detected at the Rifampicin resistance determining region of *rpoB WT7* 1 (0.7%), Isoniazid Wild type gene deletion at *katG WT* 5 (3.4%), *inhA WT1* 2 (1.4%) and *inhAWT2* 1 (0.7%). No resistance to second-line antituberculosis drugs was detected in this study. **(Table 2)**

Nucleotide changes detected by MUT probes

The highest rifampicin resistance was experienced in genes *rpoB*, which had mutant probes *rpoB MUT2A* in the codons *526 to 529.*

Table 2:
Percentages for molecular drug resistance

Percentages for First Line Drug resistance		Resistance Detected Frequency (%) n =145	Resistance Not Detected Frequency (%) n=145
GenoType MTBDR*plus*	INH Monoresistance	7(4.8)	138(95.2)
	RIF Monoresistance	5(3.4)	140(141.6)
	MDR	2(1.4)	143(98.6)

Table 3:
Specific nucleotide changes detected by MUT probes

	Mutant gene	*Mutant Probe*	*n=14*	*codons*	*Amino_acid_Change*	*Nucleotide Change*
RMP resistance	*rpoB*	*rpoB MUT2A*	4	*526 to 529*	*H526Y*	*cac> tac*
	rpoB	*rpoB MUT3*	1	*530 to 533*	*S531L*	*tcg>ttg*
MDR	*rpoB*	*rpoB MUT3,katG MUT1*	1	*530 to 533*	*S531L,S315T1*	*tcg>ttg, agc>acc*
	katG	*katG MUT1*	1	*526 to 529,315*	*H526R, S315T1*	*cac> tac,agc>acc*
INH resistance	*inhA*	*inhA MUT1*	3	*-15*	*C15T*	
	katG	*katG MUT1*	4	*315*	*S315T1*	*agc>acc*

This mutation resulted in the change of amino acid *H526Y*, changing the nucleotide from *cac> tac*. Additional rifampicin resistance was associated with probe *rpoB MUT3* in the codons *530 to 533*, this resulted in the change of amino acid *S531L*, changing the nucleotide from *tcg>ttg*. Multidrug resistance showed mutations in the genes *rpoB* and *katG*. Gene *rpoB* mutations were detected by mutant probes *rpoB MUT3*, *katG MUT1* codons *530* to *533*.

These mutations resulted in amino acid changes *S531L*, *S315T1* resulting in specific nucleotide changes tcg>ttg, agc>acc and cac> tac, agc>acc. *katG* gene was detected by mutant probe *katG MUT1*, in codons *526* to *529,315* resulting in changes in amino acid *H526R*, *S315T1* and nucleotide changes from *cac> tac,agc>acc*. High-level isoniazid resistance was expressed through mutation in the gene *inhA*, which was detected by the mutant probe *inhA MUT1*, in codon *-15*, resulting in amino acid change *C15T*. Low-level Isoniazid resistance was shown by gene *katG*, which was detected by mutant probe *katG MUT1*, codon *315*, resulting to change in amino acid *S315T1* and nucleotide change from *agc>acc* in the amino acid S315T1. **(Table 3).**

Mutation pattern in TB cases

Codon analysed in new TB cases were Codon *-15* 1 (0.7%), codon *315* 2 (1.4%), codon *526 to 529* 2 (1.4%), While in retreatment cases were codon *-15* 2 (1.4%), codon *315* 3 (2.0%), codon *526 to 529*, 4 (2.6%) and codon *530 to 533* 2(1. 4%).Amino acid change among the New Tb Cases were, *C15T* 1 (0.7), *H526Y* 1 (0.7%), *S315T1* 1 (0. 7%).While in the retreatment was *C15T* 1 (0.7%), *H526R/S315T1* 1 (0.7%), *H526Y* 3 (2.0%), *S315T1* 3 (2.0%), *S531L* 1 (0.7%), *S531L/S315T1* 1 (0. 7%). Out of as ample as 256, 145 samples were tuberculosis confirmed cases of which 32 (22.1%) were from new TB cases and 113 (77.9%) from retreatment. The total for isoniazid resistance was 7 (4.8%), out of which 2 (6.25%) were in new cases and 5 (4.4%) in retreatment cases. Molecular MDR cases were 2 (1.4%) and all were in retreatment cases. Rifampicin resistance was 5 (3.4%), 1 (3.1%) in new case and 4 (3.5%) in retreatment. **(Figure 5).**

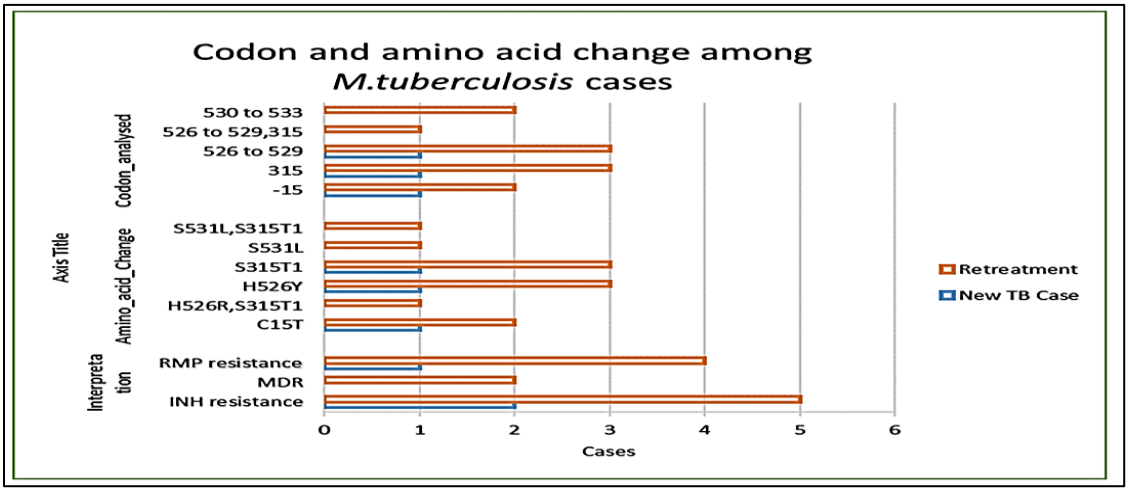

Figure 5:
Mutation patterns among TB cases

Mutant and wild-type probes by TB cases

Mutant probes in New TB cases were *inhA MUT1* 1 (3.1%), *katG MUT1* 1 (3.1%), *rpoB MUT2A* 1 (3.1%), while mutant probes in retreatment cases were *inhA MUT1* 1 (0.9%), *katG MUT1* 4 (3.5%), *rpoB MUT2A* 3 (2.7%), *rpoB MUT3* 1 (0.9%), *rpoB MUT3/katG MUT1* 1 (0. 9%).Wild type gene probes in the new TB Cases were, *inhA WT1* 1 (3.1%), *katG WT* 1 (3.1%), while in the retreatment cases were, *inhA WT1/inhAWT2* 1 (0.9%), *katG WT* 3 (2.7%), *rpoB WT7/katG WT* 1 (0.9%). **(Figure 6).**

Discussion

Out of a sample size of 256, there were more males 168(65.5%) compared to females 88 (34.4%), which is in agreement with the WHO report 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 (9). The majority of participants were aged 18 years and above 251(98%), while the remaining 5(2%), were 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 findings in this study are consistent with the findings from Makati *et al* who reported that the 31–40 years age group was the most predominant group for detection of DR-TB and the male population was higher at risk compared to their female counterparts. (18). 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 samples were from new cases, and 82.8% were from previously treated samples (19). These findings are consistent with the current study that found that the majority of TB cases were retreatment 113(77.9%) while new Tuberculosis cases were 32 (22.1%).

General isoniazid resistance was 11(7.6%) in all the cases while rifampicin was 10(6.9%) across all the TB cases. For first-line Drug resistance on culture, isoniazid showed the highest resistance at 7.6%, followed by rifampicin at 6.2% and MDR at 1.8 %.

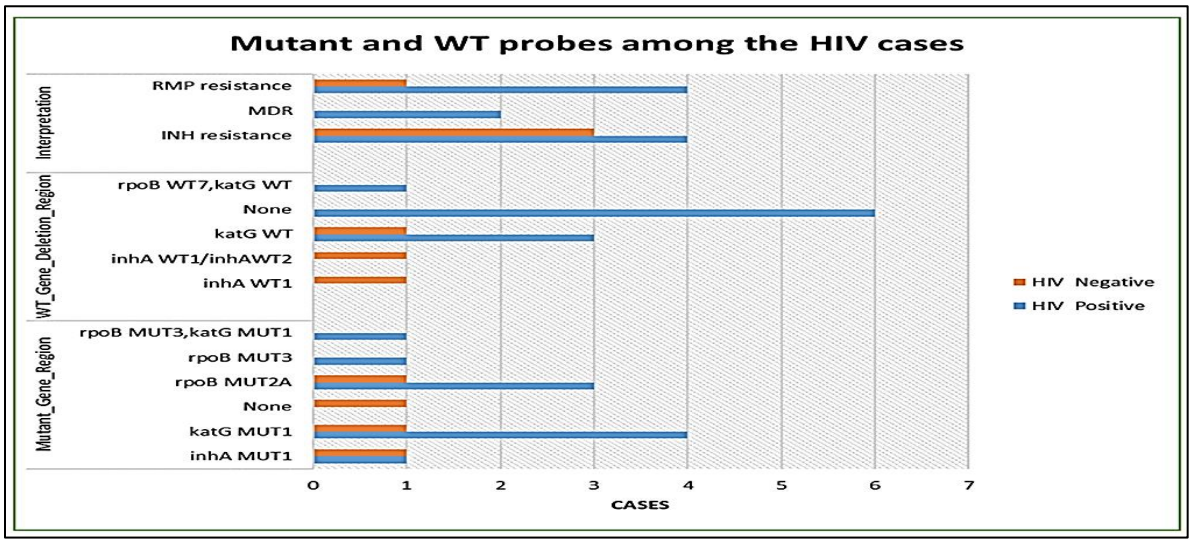

Figure 6:
Mutant and wild-type probes by TB cases

The study further found out that out of 7 that developed rifampicin resistance 2(28.6) of the isolates were MDR. No resistance was experienced by other first-line drugs, Ethambutol, Streptomycin, Pyrazinamide and second-line Fluoroquinolones and aminoglycosides. Low MDR resistance in Kisumu County was consistent with other studies in high-burden countries which encountered the same low levels (1.8%) for MDR and Ethiopia with 1.1% (20), as well as India, recorded the same percentage (21). Kidenya *et al*., also reported prevalence for the past 15 years in Tanzania ranging from 0.4–2.1%, in addition to East Africa at 0.4–4.4% (22). The MTBDR*plus* showed that isoniazid and rifampicin Monoresistance was 7(4.8%) and 6(4.1%) respectively while isoniazid and rifampicin Heteroresistance was 1(0.4%) each. All the MDR 2(1.8 %) cases were retreatment cases. This finding was consistent with a study by Saba *et al*, conducted in Pakistan one of the high-burden countries, which indicated that the retreated cases are at more risk of infection with MDR strain and they can acquire resistance to Rifampicin (RIF) or Isoniazid (INH) during the treatment course (23). Molecular Line Probe assay for isoniazid resistance was 2(6.3%) in the new cases and 7(6.2%) in retreatment cases whereas rifampicin resistance was 1(3.1%) in new cases and 6(5.3%) in retreatment cases. High INH resistant strains had mutations in the promoter region of *inhA* gene at codon *-15* with an amino acid change of *S315T1*, a similarly high prevalence (85%) of *S531L* mutation in rifampicin-resistant isolates was reported in a study in Cameroon (24), while low INH resistant strains had mutations in the *katG* gene at codon 315. Among rifampicin-resistant strains, four isolates displayed mutations at codon *526 to 529* in the *rpoB* gene with an amino acid change of *H526Y* and one isolate displayed mutation at codon *530 to 533* in the *rpoB* gene with an amino acid change of *S531L*. The MDR strains had mutations in the *rpoB* and *katG* genes. The *rpoB* gene displayed mutations at codons *530 to 533* with amino acid changes of *S531L* and *S315T1*, while *katG* had mutations at codons *526 to 529* and *315* with amino acid changes of *H526R* and *S315T1*. In a study conducted by Gagneux and others, they postulated that the presence of a high frequency of *S531L* mutation around the globe might be due to its strong selection in the environment and transmissibility (25). In the current study, isoniazid resistance was shown by high mutations in the *inhA* promoter showing high-level resistance and in the *katG* showing low-level isoniazid resistance. Most reports suggest that resistance of MTB to INH shows mutation at codon 315 (7). Findings from this study were similar showing 100% of all isolates had mutations at *S315T1*, attributed to a high level of drug resistance to INH. The highest proportion of rifampicin resistance was experienced in probes *rpoB MUT2A* which had mutations in the codons *526* to *529.* A significant number of studies report that resistance of Mycobacterium tuberculosis to Isoniazid shows mutation at codon *315* (26). These mutations resulted in amino acid changes *S531L, S315T1* resulting in specific nucleotide changes *tcg>ttg, agc>acc* and *cac> tac,agc>acc*. The current study showed that RIF resistant isolate had the mutation in the amino acid *S531L*; the most often recorded resistance mutation in various countries (13). Studies show that *katG* is the most common region targeted with a bulk of mutations occurring in codon 315 in 30–90% of INH resistant strains, in the current study, low-level Isoniazid resistance was exhibited in the gene loci *inhA MUT1* and high-level Isoniazid resistance was shown in loci *katG MUT1*. A greater variability was observed in amino acid changes in retreatment cases compared to new cases. This may be an indication that such mutations might be acquired during treatment

courses by repeated administration of the same anti-TB drugs.

Study limitations

There were limitations to the generalization of LPA results. Although LPA can detect the mutations that are most frequently identified in resistant strains, some mutations that confer resistance are outside the regions covered by the test and therefore resistance cannot be completely excluded (inferred) even in the presence of all WT probes. Thus, in some cases, additional phenotypic DST may be necessary to provide a full assessment of results.

Conclusion

The findings of this study showed a high incidence of Isoniazid resistance and this implies that mutations that are responsible for resistance to INH in Kisumu County are accruing and an added increase of rifampicin resistance will ultimately lead to MDR-TB. A greater variability was observed in amino acid changes among isoniazid and rifampicin resistance in retreatment cases compared to new cases and additional mutations were more associated with HIV positive cases compared to HIV negative cases. No mutations were detected against ethambutol, pyrazinamide, streptomycin and Second line anti-tuberculosis drugs. Various Wild Type mutations were detected in this study, implying that the scope of mutations that confer resistance to MTB may be much more comprehensive than those depicted in the research therefore gene mutations observed from the use of the different drug regimens may give more insight on some of the reported geographical variances in drug efficacy. This is useful in understanding drug-resistant gene migrations within populations since the frequency of mutations varies geographically.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests

All authors declare that they do not have any competing interests.

Authors' contributions

The study was conceived by OF, OR, OB, OA, NJ, GB and WS. OF wrote the initial draft with input from all authors. OF finalized the manuscript and all authors reviewed and approved the final version.

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