Characterisation of Drug-Resistant Strains of *Mycobacterium Tuberculosis* Isolated from TB and HIV Co-Infected Patients Attending Chest Clinic at Kericho County Referral Hospital, Kenya

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Abstract

Background: Tuberculosis-HIV (TB-HIV) co-infection remains a leading cause of AIDS-related mortality. HIV-induced immunosuppression increases TB susceptibility and risk of treatment failure when drug-resistant strains occur, making MDR-TB surveillance critical. This study aimed to characterise multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB) strains among TB-HIV co-infected patients at the Chest Clinic of Kericho County Referral Hospital, Kenya.

Methods: A hospital-based cross-sectional study was conducted among patients receiving highly active antiretroviral therapy (HAART) at the Chest Clinic of Kericho County Referral Hospital between February and September 2024. A total of 174 sputum samples were collected from TB-HIV co-infected patients. Phenotypic and genotypic assays were used to characterise MDR-TB strains and assess their genetic diversity. Statistical analysis was performed using STATA version 18, incorporating inferential tests to evaluate diagnostic agreement and linear regression to identify predictors of drug resistance mutations.

Results: Of the 174 screened patients, 156 were enrolled, comprising 113 females (72.4%) and 43 males (27.6%). Among the HIV-infected patients, *Mycobacterium tuberculosis* was the predominant species (80.6%, n = 88), while non-*tuberculous mycobacteria* accounted for 19.4% (n = 25) of isolates. Of the 95 MTB isolates, 19 (20%) exhibited resistance to at least one first-line drug: five (5.3%) to isoniazid, six (6.3%) to rifampicin, and eight isolates were classified as MDR-TB (resistant to both INH and RIF). Resistance-associated mutations were detected in katG and inhA (INH resistance) and rpoB (RIF resistance). Lineage 4 (71.3%) was the most prevalent, followed by Lineage 3 (23.8%).

Conclusion: High female enrollment may reflect gender differences in healthcare access or disease burden. The 20% resistance rate among MTB isolates, including 8.4% MDR-TB with well-characterised mutations, is concerning. Findings highlight the need for enhanced drug susceptibility testing and tailored strategies in high-burden settings.

Keywords**:** *Mycobacterium Tuberculosis Complex, Multidrug Resistance, Genetic Diversity, Immunocompromised, Characterization*

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Introduction

Infection with both HIV and TB, commonly referred to as HIV-TB co-infection, presents a significant and complex health challenge. TB tends to affect HIV-positive individuals more severely than those without HIV and is a leading cause of death among this population (1). The interplay between HIV and TB can substantially reduce life expectancy if left untreated (2), similar to other opportunistic infections. Research shows that women and girls

are disproportionately affected, accounting for 53% of the HIV-infected population (3).

Despite advances in TB and HIV therapies, co-infection remains a major global health concern. In 2023, the TB incidence was 134 per 100,000 population, with 6.1% of cases occurring in people living with HIV (4). The annual risk of developing active TB through reactivation of latent infection in untreated HIV-positive individuals is estimated at 3–16% (5). In 2021, out of approximately 9 million global TB cases, 13% were co-infected with HIV. TB alone caused about 1.5 million deaths, excluding those from HIV-TB co-infection.

The Global TB Report estimated 167,000 HIV-TB deaths in 2022, while the WHO reported 190,000 deaths in 2021, 11% of which were in children. HIV-TB co-infection accounts for roughly 30% of AIDS-related mortality (6). The severity of TB in HIV-positive individuals, especially among women and children, highlights the urgent need for more research into the spread, identification, and management of multidrug-resistant (MDR) TB strains in this population (7,8). Addressing this gap is essential for improving patient outcomes, guiding public health interventions, and advancing global TB and HIV eradication goals.

This study aimed to determine the presence and characteristics of MDR strains of *Mycobacterium tuberculosis* by using phenotypic DST and genotypic assays (LPAs) to identify resistance-conferring mutations in HIV-TB co-infected patients attending the Chest Clinic at Kericho County Referral Hospital, Kenya.

Material and Methods

Study area

The study was conducted at Kericho County Referral Hospital (KCRH), located in Kericho County in Kenya.

Study design

An analytical cross-sectional study was conducted to collect quantitative data from patients on HAART attending the Tuberculosis (TB) Clinic at KCRH between February and September 2024.

Sample size and sampling

The sample size consisted of 174 sputum samples, calculated using Cochran's formula:

$(n = (Z^2 * p * (1 – p)) / E^2)$ based on the assumption that 13% of individuals co-infected with HIV and TB were also infected with TB (9, 10).

A simple random sampling technique was employed. The sampling frame included all patients diagnosed with both TB and HIV during the study period, identified from TB/HIV clinic registers and laboratory records. Random selection was performed by assigning each eligible patient a unique code and using a computer-generated random number sequence to select 174 participants.

Selected individuals were approached during routine clinic visits, informed about the study, and recruited upon providing written consent. Patients who declined participation were replaced by the next randomly selected individual to maintain the sample size.

$$N = \frac{(Z)^2 .P.(1-P)}{(E)^2}$$

Where:

n = Required sample size

Z = Z-score corresponding, to the desired confidence level =1.96

p = Estimated proportion of the population = 0.13 (13 %)

1-p= 1-0.13 =0.87

E = Margin of error =0.05

$$N = \frac{(1.96)^2\ 0.13(1-0.13)}{(0.05)^2} = 174$$

Study population

The study focused on patients co-infected with HIV and tuberculosis who were receiving care at HAART clinics and concurrently attended the Chest Clinic at Kericho County Referral Hospital (KCRH).

Inclusion criteria. All adult patients co-infected with HIV and TB, presenting with clinical symptoms suggestive of pulmonary TB, and willing to provide written consent were included in the study. For participants unable to provide consent, an assent form was used. Any

individual capable of expectorating sputum was considered eligible.

Exclusion criteria. Patients who were unable to provide adequate sputum specimens or those who were severely ill were excluded from the study.

Data collection

Consent administration. Participants were informed about the purpose of the study and the procedures involved, and were assured that participation posed no risk. They were given the right to choose whether or not to participate by signing a consent form, or an assent form in the case of minors. Participants were made to understand that participation was voluntary and that they were free to withdraw at any time without providing a reason and without incurring any cost.

Questionnaires administration. A short questionnaire was administered to patients to obtain information on TB preferences and the characterisation of TB strains among TB/HIV co-infected individuals. The data collected aimed to improve the management of HIV/TB co-infected and TB mono-infected patients and to support efforts in controlling drug-resistant TB strains.

Patients were politely requested to complete the questionnaire, which was designed purely for academic purposes. They were asked to mark (X) where applicable for items such as:

- Gender
- Date of birth/Age
- Marital status
- Have you ever been diagnosed with TB?
- Which type of TB do you have?
- Are you currently on TB treatment?
- Have you had X-ray examinations?
- Have you ever been diagnosed with HIV/AIDS?
- What is your HIV status?
- Are you on ARVs?

Participants were thanked and appreciated for their cooperation.

Sputum sample collection and storage

Each participant was provided with a clean, sterile, dry, wide-mouthed, leak-proof screw-capped container (50 ml Falcon tube). Participants were instructed on proper sputum collection: breathe in deeply, hold the breath for about 5 seconds, exhale slowly, repeat this process, then perform a deep, forceful cough to bring up sputum from the lungs. The sputum was then expectorated directly into the container. This process was repeated until at least 5 ml of sputum was obtained. The samples were transported to the KCRH laboratory and stored under refrigeration at 2–8 °C until analysis was performed.

Laboratory procedures

A phenotypic technique was used to process sputum samples that tested positive for Alcohol-Acid Fast Bacilli (AAFB) by microscopy and GeneXpert, using the N-acetyl-L-cysteine–sodium hydroxide (NALC-NaOH) method. The samples were inoculated into BD BACTEC MGIT 960 for 42 days and onto Lowenstein-Jensen egg medium slants at 37 °C for up to 8 weeks, with weekly monitoring for growth.

An immunochromatographic assay (BD MGIT TBc ID test device - TB Ag MPT64) was used to rapidly identify *Mycobacterium tuberculosis* complex from liquid culture, and AFB fluorescent microscopy staining (FM) was performed to confirm the growth of MTBc isolates. All procedures followed the Revised National Tuberculosis Control Program (2023) Standard Protocols (11).

Phenotypic drug susceptibility testing (DST) was performed on *M. tuberculosis* isolates for first-line drugs: streptomycin (STR), isoniazid (INH), rifampicin (RIF), ethambutol (EMB), and pyrazinamide (PZA), using the automated BD BACTEC MGIT 960 growth detection system (BD, Sparks, MD, USA). Final drug concentrations in MGIT media were: STR 1.0 µg/mL, INH 0.1 µg/mL, RIF 1.0 µg/mL, EMB 5.0 µg/mL, and PZA 100 µg/mL, as per kit instructions.

Genotype® MTBDRplus v.2.0-line probe assays (LPAs) (Hain Life Science, Nehren, Germany) were used to detect genetic mutations in the *rpoB* gene (conferring RIF resistance) and *katG* and *inhA* genes (conferring INH resistance).

These tests were performed on 95 culture-positive *M. tuberculosis* clinical isolates. MDR-TB isolates (resistant to both INH and RIF) were analysed to identify mutations associated with resistance. LPA procedures included genomic DNA extraction, multiplex PCR amplification, reverse hybridisation, and interpretation of results using DNA strip technology (12).

Data collection instruments

The study utilised both clinical observation and experimental methodologies for data collection. Clinical data included demographic information, medical history, and ART status, while laboratory data comprised sputum samples collected for culture, drug susceptibility testing (DST), and molecular analysis.

Validity

A pilot study was conducted to test and retest methodologies, ensuring the reliability and validity of the tools. Instrument pre-testing was undertaken to eliminate ambiguities and confirm that respondents provided data aligned with the study's objectives. Additionally, all laboratory procedures adhered to standardised protocols.

Reliability

. To guarantee reliability, a pre-test was conducted on the research instruments to confirm their reusability. Additionally, quality control measures were implemented for all tests and procedures during each run to ensure accuracy and consistency.

Data management

Data Entry and Cleaning: Quality controls were performed on all laboratory and clinical data using standardised data collection forms. The data were manually double-checked for completeness, accuracy, and consistency, and entered twice independently in accordance with the SOPs. Access to data was restricted based on roles and responsibilities. A secure, backed-up database was maintained, and all data were de-identified to protect participant confidentiality.

Quality control

All laboratory analyses were conducted in accordance with standard operating procedures by well-trained laboratory personnel. Each test run included both negative and positive controls. Molecular-grade water and the *Mycobacterium tuberculosis* H37Rv reference strain (ATCC 27294), which is sensitive to all drugs tested, were used to ensure accuracy and reliability.

Statistical data analysis

Data analysis and management were performed using Microsoft Excel, with descriptive statistics used to present the findings. STATA version 18 was employed for inferential analyses, including Cohen's Kappa coefficient to assess agreement between tests, linear regression to evaluate predictors of drug mutations, and Firth's penalised likelihood logistic regression to identify predictors of specific drug mutations. This model was selected due to sparse data and to minimise small sample size bias. A p-value of < 0.05 was considered statistically significant at a 95% confidence interval.

Ethical considerations

Ethical approval was obtained from the University of Kabianga Institutional Scientific and Ethics Review Committee and the Kericho County Referral Hospital Ethics Board Committee, with reference numbers (ISERC/2024/006) and (KCO/REC/2024/05/01), respectively. Additionally, the study was approved by the National Commission for Science, Technology, and Innovation (No. 803742-NACOSTI/P/24/35663).

Informed consent was obtained from all willing participants before enrolment. For participants under the age of 18, both parental consent and child assent were sought. All data were anonymised and restricted to ensure confidentiality.

Results

Demographic characteristics

A total of 174 Chest Clinic attendees were screened for tuberculosis (TB), of which 156 were enrolled in the study. Among the enrolled participants, 113 (72.4%) were female, and 43 (27.6%) were male. The ages of participants ranged from 18 to 64 years, with

most falling between 25 and 50 years. The mean age of the study population was 36 years.

Mycobacterium species identification

The study confirmed the presence of *Mycobacterium* species in 104 (80.6%) isolates from 129 culture samples. Of these, 95 (73.6%) were identified as members of the *Mycobacterium tuberculosis* complex (MTBC), comprising 88 isolates of *M. tuberculosis* (68.2%), 3 isolates of *M. africanum* (2.3%), and 4 isolates of *M. bovis* (3.1%).

Non-tuberculous mycobacteria (NTM), also referred to as Mycobacteria other than tuberculosis (MOTT), accounted for 25 isolates (19.4%). These included *M. avium* (3 isolates, 2.3%), *M. abscessus* (4 isolates, 3.1%), *M. kansasii* (5 isolates, 3.9%), *M. ulcerans* (2 isolates, 1.6%), *M. fortuitum* (7 isolates, 5.4%), and *M. intracellulare* (4 isolates, 3.1%).

Additionally, 9 isolates (6.9%) failed amplification and could not be fully characterised.

Phenotypic culture results and agreement analysis

Of the 129 positive culture samples (82.7%), 25 isolates (19.4%) were identified as *Mycobacterium other than tuberculosis complex* (MOTT), also known as non-tuberculous mycobacteria (NTM) or atypical mycobacteria. The remaining 104 isolates (80.6%) were confirmed as *Mycobacterium tuberculosis complex* (MTBC), and only 95 (73.6%) MTBC isolates were subjected to drug susceptibility testing.

To assess agreement between the Mycobacteria Growth Indicator Tube (MGIT) system and culture on Lowenstein-Jensen medium, Cohen's Kappa statistic was computed. The observed agreement was 88.5%, with a Kappa coefficient of 0.6663, indicating substantial agreement.

MTB complex (MTBc) Vs. *Mycobacterium* other than *Mycobacterium* tuberculosis (MOTT)

Of the 129 culture-positive samples, 104 (80.6%) were identified as *Mycobacterium tuberculosis complex* (MTBC), while 25 (19.4%) were classified as *Mycobacteria other than tuberculosis* (MOTT).

Drug resistance analysis

Out of the 95 sputum samples that tested positive for *Mycobacterium tuberculosis* (MTB), 20% (n = 19) were resistant to at least one drug. Resistance was observed either to Isoniazid (INH), Rifampicin (RIF), or as multidrug-resistant TB (MDR-TB).

The association between patient type (new presumptive, relapse, and follow-up) and drug resistance category (mono-drug resistance to INH, mono-drug resistance to RIF, MDR-TB, and drug-sensitive cases) was assessed.

Rifampicin resistance assessment by molecular GeneXpert MTB/RIF-Ultra assay

To assess *Mycobacterium tuberculosis* resistance to Rifampicin (RIF), genotypic testing was performed using GeneXpert (GXP). Of the 156 enrolled patients, 91 (58.3%) tested positive for MTB by GXP. Among these GXP-positive cases, Rifampicin resistance (RR) was detected in 13 isolates, representing 8.3% of the total.

Genetic diversity of MDR-TB strains

The genetic diversity of MDR-TB strains at Kericho County Referral Hospital was analysed across several factors: geographical location (sub-county), patient type, gender, age distribution, and resistance pattern.

Table 1:
Results* of the *Phenotypic Analysis

Mycobacterium	Total
Phenotypic Culture Positive	129(82.7%)
MTB Complex (MTBC)	104 (80.6%)
Mycobacterium other than TB (MOTT)	25 (19.4%)
Drug susceptibility testing(DST)	95 (73.6%)
Inter MGIT/LJ Culture Agreement	Kappa= 0.6663

A total of 129 sputum samples tested positive for MTBDRplus using the molecular GenoType® assay. Of these, 98 (76%) were female, and 31 (24%) were male. Among the 84 isolates of the *M. tuberculosis* complex, 88.4% were sensitive to both rifampicin and isoniazid, while 20% (n = 19) were resistant, and 6.9% (n = 9) failed amplification.

Of the 19 drug-resistant MTB isolates, 73.7% (n = 14) were from female patients. Resistance patterns included mono-resistance to rifampicin (6 isolates, 31.6%), mono-resistance to isoniazid (5 isolates, 26.3%), and resistance to both RIF and INH (8 isolates, 42.1%).

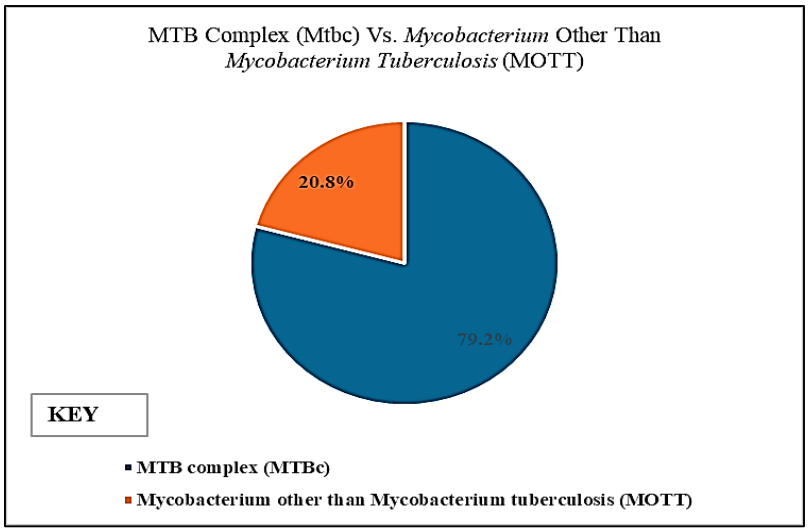

Figure 1:
The distribution between MTBC vs. MOTT

Table 2:
Drug Resistance Patterns

Type of Patient	Total	Drug Resistance	Percentages (%)
Follow-up cases	2	2	100%
New Presumptive cases	76	11	14.5%
Relapse cases	17	6	5.9%
Total	95	19	

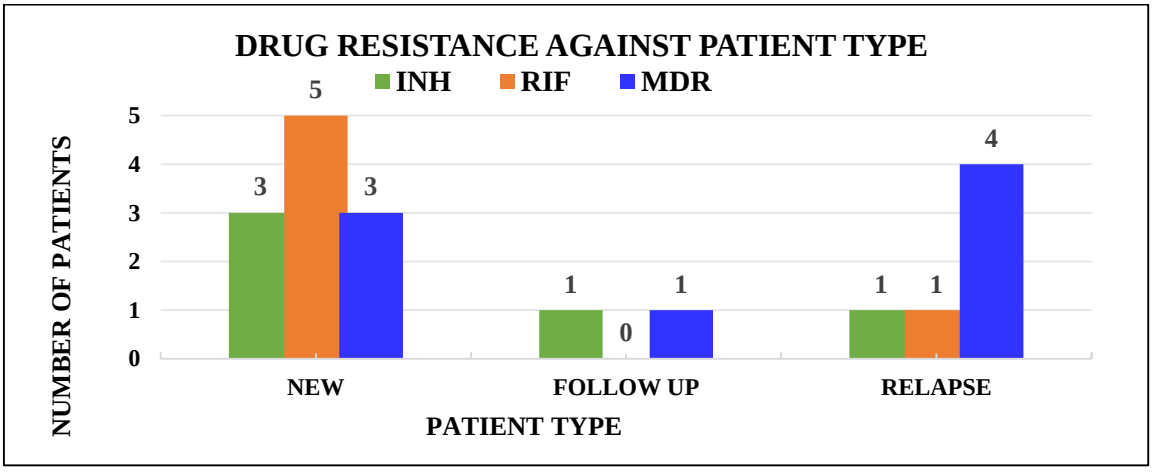

Figure 2:
Type of Drug Resistance associated with patient types

MDR-TB was most common among adults aged 20–40 years, INH resistance occurred across all age groups, and RIF resistance was predominantly observed in patients aged 30–40 years.

Drug resistance by patient type

New presumptive cases showed a mix of mono-resistance and MDR-TB. Relapse cases are more likely to have MDR-TB, indicating possible failure or re-infection. Follow-up cases also had a single case of multidrug-resistant tuberculosis. New presumptive cases exhibited a mix of mono-drug resistance and multidrug-resistant TB (MDR-TB).

Relapse cases were more likely to present with MDR-TB, suggesting possible treatment failure or re-infection. Follow-up cases included a single instance of MDR-TB.

The MTBDRplus assay found the resistance genes with *inhA* and *katG* for isoniazid resistance and *rpoB* for rifampin resistance. There was a mutation with codon 315 (S315T) and a WT probe in the *katG* gene, as well as a mutation with codon C15T and a WT1 probe in the *inhA* promoter.

The mutations to *KatG* S315 T1, *inhA*, and *rpoB* S531L caused resistance to RIF/INH. Of these mutations, eight (42.1% of all cases) were found in MDR-TB. Six cases (31.6%) had S315L MUT3 mutations in the *rpoB* gene, causing resistance to RIF.

Five cases (26.3%) had mutations in the *KatG* S315 T1, *inhA*, causing resistance to INH. *Mycobacterium tuberculosi*s (MTB) strains show a mixed genetic diversity, with the L4 (71.3%) lineage being the most prevalent, followed by L3 (23.8%), which is the second most common lineage (Table 3).

Discussion

Most patients were younger females, which may reflect high TB-HIV co-infection rates among individuals in economically productive age groups. Alternatively, a higher proportion of women may have been selected, introducing potential selection bias. Previous studies show women in high HIV prevalence regions face increased TB risk due to social, economic, and geographic barriers to healthcare (13). A Tanzanian study reported HIV-positive women were 1.5 times more likely to develop drug-resistant TB (DR-TB) than men, corroborating our findings, while research from Nigeria found older males were more likely to develop DR-TB (14, 15).

Globally, *Mycobacterium tuberculosis* (MTB) is the most prevalent pathogen among HIV-positive TB patients, and this study confirmed a similar trend. Studies from South Africa report MTB in about 95% of TB cases among HIV-positive individuals (16). WHO estimates MTB accounts for nearly 90% of TB cases in HIV-positive populations worldwide, making TB a leading cause of death in this group (4, 17).

Other *Mycobacterium* species were detected in low proportions. *M. africanum* and *M. bovis*—known TB agents in West Africa have lower transmission and virulence than MTB (18). *M. bovis* (4.6%), a zoonotic species spread through contact with infected animals or unpasteurized dairy, occurs sporadically among HIV-positive individuals, especially those in close contact with livestock, such as in Kericho County (19, 20).

Table 3:
Mutation Detection in MTB Drug Resistance-Related Gene

Type of resistance	Codon	Common Mutation	Gene Type	Lineage	Resistant
RIF/INH Resistant	S315TL, S513L, C15T	Ser531Leu,His526Tyr ,Ser315Thr,-15C-T	*rpoB, katG, and inhA*	L4/L3	8(42.1%)
RIF mono-Resistant	S513, S526L, S516L	Ser531Leu,His526Tyr	*rpoB*	L4/L3	6(31.6%)
INH mono-Resistant	S315TL	Ser315Thr,-15C-T (promoter)	*inhA* and *katG*	L4/L3	5(26.3%)

Detection of *M. bovis* (3.8%) highlights the need for zoonotic TB surveillance and treatment adjustments due to pyrazinamide resistance. Similarly, *M. africanum* (2.9%) may require molecular diagnostics for accurate detection (21–23). Non-tuberculous mycobacteria (MOTT) infections are more common in immunocompromised individuals. HIV-positive patients are up to five times more likely to contract MOTT (24). A South African study found MOTT accounted for 20% of TB diagnoses among HIV-positive individuals (25). Since MOTT infections require different drug regimens, incomplete species characterisation before treatment can lead to mismanagement (26).

Among MDR-TB isolates, the most frequent mutation linked to rifampicin (RIF) resistance was at codon S315TL, followed by isoniazid (INH) resistance at codon S513L. INH resistance is mainly caused by katG and inhA mutations. HIV-positive patients in Mumbai showed high RIF and INH resistance prevalence (27). HIV-TB co-infection may increase the risk of drug resistance due to repeated TB exposure and weakened immunity. INH resistance often signals MDR-TB, particularly in HIV-positive patients, due to poor adherence or katG/inhA mutations. A Tanzanian study reported INH mono-resistance in 28% of TB-HIV co-infected patients (28). Though less frequent, RIF mono-resistance is critical as it often precedes MDR-TB, complicating therapy. HIV patients are highly vulnerable to MDR-TB, defined by resistance to both INH and RIF due to immunosuppression, leading to poorer outcomes (29). In South Africa, HIV-positive individuals had a 50% higher MDR-TB risk than HIV-negative TB patients (30). These findings underscore the need for frequent resistance testing and tailored treatment plans, particularly for rpoB, katG, and inhA mutations common in Lineage 4 (L4) and Lineage 3 (L3) strains (33).

Several new presumptive TB cases were identified, indicating primary transmission of resistant strains, as seen in Uganda (13). Relapse cases with resistance suggest treatment failure or reinfection. The high proportion of Non-MTB/Other species may reflect diagnostic challenges or post-treatment complications (31). Among follow-up patients, the absence of drug-sensitive cases and presence of resistance indicate treatment failure or acquired resistance, requiring second-line regimens and closer monitoring. Although baseline cases were few, the presence of only drug-sensitive TB at baseline is reassuring.

Study limitations

This study focused on 174 patients from Kericho Referral Hospital, which may not be representative of the broader population or regions with different TB/HIV dynamics. Additionally, the lack of Whole-Genome Sequencing (WGS) limited our ability to comprehensively assess genetic variations associated with drug resistance and the genetic diversity of MTB strains.

Conclusion

This study underscores the significant burden of TB-HIV co-infection and drug resistance in Kericho County. The predominance of younger female patients may indicate a higher co-infection risk among economically active women or reflect gender-related disparities in healthcare access, though selection bias cannot be ruled out. Mycobacterium tuberculosis (MTB) was the most prevalent species among HIV-positive TB patients, reaffirming its dominant role in morbidity and mortality within this vulnerable group at KCRH. Non-tuberculous mycobacteria (MOTT) infections, while less frequent, remain clinically significant in immunocompromised individuals, as misdiagnosis can lead to inappropriate therapy. The study also revealed a concerning prevalence of multidrug-resistant TB (MDR-TB), with common mutations in rpoB, katG, and inhA genes, and evidence of both primary transmission and acquired resistance. These findings emphasise the importance of routine drug susceptibility testing, adherence monitoring, and tailored treatment strategies. The presence of resistant strains among new cases signals ongoing

community transmission, while relapse cases highlight gaps in treatment success.

Recommendations

This study highlights the high burden of TB-HIV co-infection and drug resistance in Kericho County Hospital and proposes strategies to improve TB management among HIV co-infected patients. Key recommendations include strengthening diagnostic capacity through routine molecular testing for species identification and resistance detection, enhancing MDR-TB surveillance and treatment through regular drug susceptibility testing and adherence monitoring, and addressing gender and socioeconomic barriers by improving healthcare access for women and economically active age groups. Additionally, integrating HIV and TB programs for joint screening, treatment, and follow-up is essential, along with investing in research and monitoring to confirm species distribution, resistance patterns, and transmission dynamics.

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Authors' contributions

- Roseline Vicky Bosibori: Conception and design of the study, data collection, laboratory analysis, interpretation of results, and preparation of the manuscript.
- Ibrahim Daud and Janeth Kombich contributed to the study design and manuscript review. All authors read and approved the final manuscript.

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