Genetic Mutations and Antibiotic Resistance Patterns in Non-Tuberculosis Mycobacterium Isolates from HIV-1 Patients in Western Kenya

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ABSTRACT

Primarily among immunocompromised people, notably those living with HIV/AIDS, antimicrobial resistance (AMR) among non-tuberculous mycobacteria (NTM) has become a major public health issue. Though the main pathogen in such populations is Mycobacterium tuberculosis, the importance of NTM in causing AMR and complicating treatment plans is becoming more well known. Still, the causes of medication resistance in NTM especially in relation to HIV co-infection remain mostly unknown. This study sought to ascertain the antimicrobial susceptibility patterns of NTM isolates and identify genetic alterations linked with isoniazid and rifampicin resistance among HIV-1 infected and uninfected patients in Western Kenya. Adult HIV-1 infected individuals showing suspected pulmonary tuberculosis were subject to a cross-sectional analytical laboratory analysis. Samples of sputum were gathered; NTM isolates were grown and identified. The broth microdilution technique was used for antimicrobial susceptibility testing. Line probe tests aiming at the rpoβ, katG, and inhA genes helped to find genetic alterations linked to medication resistance. Of 167 participants, 59 NTM isolates were found; most often occurring species were M. intracellularae and M. fortuitum. Observed in 12.1%, 15.2%, and 15.2% of isolates respectively were resistance to isoniazid, rifampicin, and streptomycin. HIV-positive individuals had more frequent mutations in the rpoβ, katG, and inhA genes; medication resistance and HIV status had clear correlation. The study emphasises how different treatment resistance patterns and genetic alterations cause NTM infections in HIV-positive patients to be difficultly managed. Especially in resource-limited environments, these results highlight the importance of customised treatment plans and continuous monitoring of AMR in NTM.

Keywords: Antimicrobial Drug Resistance, Non-Tuberculous Mycobacterium, Isoniazid, Rifampicin

I. INTRODUCTION

Antimicrobial resistance (AMR) among non-tuberculous mycobacteria (NTM) has developed to be a key public health concern of this century (Bryant et al., 2016; Faverio et al., 2016). AMR is a threat to effective treatment of pathogenic microbes. Therefore, sentinel surveillance for AMR markers in both pathogenic and non-pathogenic microbes is important. NTM being abundant microorganisms in nature pose a threat of spreading drug resistance traits by their interaction (Munita & Arias, 2016). Previous evidence has revealed the role that NTM have played in escalating antimicrobial resistance (Johansen et al., 2020). However, the mechanisms by which NTM spread AMR are not fully understood. But some of the inherent characteristics in NTM that are believed to be capable of decreasing drug uptake that eventually causes resistance to antibiotics include, their thick, impermeable cell walls, their presence in biofilms and granulomas (Bryant et al., 2016; Luthra et al., 2018). Additionally, some NTMs express proteins that specifically target antibiotics that consequently reduce drug efficacy (Nessar et al., 2012). It is also crucial to map genotypic and allelic variations connected to AMR at the molecular level. For instance, plasmids are used by bacteria to reproduce, therefore there could be possibilities that resistant features will be introduced into the genomes of previously vulnerable NTM by plasmids (Morgado et al., 2017; Tagini et al., 2021).

Conditions that cause immune deficiency in people includes cancer, organ transplant, HIV and AIDS and some genetic diseases (Sharma & Upadhyay, 2020; Winthrop et al., 2020). HIV and AIDS is the most prevalent cause of immune deficiency (Agizew et al., 2017). Globally, people are living with HIV and with the advent of antiretroviral

therapy, fewer number of patients with AIDS are being recorded (Lapinel et al., 2019). Previous studies have attributed the global spread of NTM to HIV infected individuals. Nevertheless, *Mycobacterium tuberculosis* (MTB) has been for a long time reported as the most prevalent opportunistic infection in patients with HIV and AIDS (Peters et al., 2019). However, dearth research on the epidemiology of NTM in HIV and AIDS patients has led to underestimation of its prevalence within TB endemic countries such as Kenya (Kaguthi et al., 2019). The situation is further compounded by antibiotic resistance in HIV patients co-infected with NTM. Although, several NTM species are now recognized as a major infective threat in HIV and AIDS individuals, their in-depth genomic investigation has not been carried out systematically (Yeung et al., 2016). Altogether, these studies seem to suggest that molecular characterization of NTM needs to be done in the context of AMR in HIV and AIDS patients.

MTB is the most important bacteria in the genus mycobacteria. Most studies on AMR have focused on MTB with a number of mutations in specific markers being described in the context of isoniazid and rifampicin resistance. Of the genes commonly analyzed for isoniazid and rifampicin resistance in MTB include *rpoβ*, *katG* and *inhA*. These genes are relatively conserved across other species in mycobacterium genus (Kim et al., 2019; Orgeur et al., 2024). Therefore, determined antimicrobial susceptibility patterns and characterized the various gene mutations in NTMs isolates associated with isoniazid and rifampicin resistance.

II. MATERIALS AND METHODS

Study design and population

A cross sectional analytical laboratory study design was used targeting adult HIV-1 infected patients presenting with presumptive pulmonary TB at Bungoma County Referral Hospital comprehensive care clinic in a resource limited setting in western Kenya. *Inclusion criteria:* HIV-1 positive presenting with TB-like symptoms including chronic productive cough lasting more than 2 weeks, loss of appetite, fever, fatigue, headache and night sweats and who consented. *Exclusion criteria*: Patients on TB treatment were excluded from the study.

HIV-1 diagnosis. Confirmation of HIV-1 was done using rapid immunochromatographic test kit, Determine™ (Abbott Laboratories, Tokyo, Japan) and first response™ (Trinity Biotech Plc, Bray, Ireland). In accordance with the Kenyan national HIV testing algorithm, participants were considered HIV-1 infected if they had HIV positive results for Determine and HIV-1 positive results using first response kits.

Smear microscopy. Sputum samples were screened by fluorescent microscopy using Auramine O stain and smears found to be positive were confirmed by light microscopy using Ziehl-Neelsen's stain as per the standard protocols of both staining methods. Sputa were graded for positivity of AFB as per the guidelines, decontaminated according to standard guidelines and divided into two parts.

Culture. Decontaminated samples of those sputum samples that were found to have no members of MTB complex were cultured on Löwenstein-Jensen (LJ) media as per the standard protocol and incubated at 37°C for a maximum of 8 weeks. Any strain of AFB grown from these samples was first biochemically identified according to manufacturer's guidelines and an rRNA based DNA hybridization assay (Accuprobe® System; Gen-Probe Inc., San Diego, CA, USA) was performed to detect the presence of MTB complex, if any.

Line probe assay for NTM. The strains negative for MTB complex were confirmed as NTM by negative niacin accumulation test, growth on paranitrobenzoic acid (PNB) incorporated in LJ media (LJ-PNB), positive catalase test and a negative result of a ribosomal RNA based DNA hybridization assay for *Mycobacterium tuberculosis* complex (Accuprobe® System Gen-Probe Inc., San Diego, CA, USA). DNA was extracted from these NTM using GenoLyse®, VER1.0 (Hain Lifescience, GmBH, Nehren, Germany) according to the manufacturer's instructions. Line probe assay for NTM was carried out using GenoType® *Mycobacterium* common mycobacteria (CM), VER 1.0 (Hain Lifescience, GmBH, Nehren, Germany) to identify the NTM as per the manufacturer's guidelines.

If NTMs were detected in a sputum sample, a request was made to the treatment providers to organize to send three consecutive sputum samples from the patient in order to understand whether there was an NTM infection according to the established American Thoracic Society (ATS) criteria. Smear microscopy, culture and LPA were then again carried out as described above.

Determination of minimum inhibition concentrations (MICs). The broth microdilution method was used to determine the minimum inhibitory concentration of the antibiotics for the NTM isolates, and the results were interpreted in accordance with the guidelines provided by the Standard Clinical and Laboratory Standards Institute (CLSI) (Brown-Elliott & Woods, 2019). A commercial radiometric medium made by Johnston Laboratories was utilized in the broth dilution technique, and the BACTEC 460-TB instrument was used to measure the CO_2 released as a result of the growth of NTM isolates in the 7H12B medium. The following concentrations of the different drugs were tested; streptomycin (STR): ≤0.5, 1, 2, 4, 8,16 and ≥64 µg/ml; isoniazid (INH), ≤0.25, 0.5, 1, 2 ,4, 8, and 8≥ µg/ml; rifampicin (RIF), ≤0.12, 0.25, 0.5, 1, 2, 4, 8, and ≥64µg/ml; ethambutol (EMB), ≤0.5, 1, 2, 4, 8 and ≥16 µg/ml.

An appropriate amount of antibiotic stock was added to Middle Brook 7H9 broth, which already contained 100 mL of oleic acid/dextrose/catalase (OADC) growth supplement and 2 ml of glycerol, in order to obtain the necessary dilution (Figure 3.1). To make a solution for well injection, growing colonies were extracted from the LJ-PNB medium and used at a concentration of 1.5×10^5 colony-forming units (0.5 McFarland standard). In 96-well microtiter plates, 100µl of 7H9 medium containing OADC was distributed. Serial concentrations were created for each antibiotic, followed by addition of 100 µl of bacterial suspension to each well. Parafilm and zip lock bags were employed to keep the microplates from drying out during the 2-week incubation period at 37℃ (Brown-Elliott & Woods, 2019). The MIC is the lowest amount of the antibiotic required to fully stop the NTM from growing (Inderlied et al., 1987). The susceptibility was determined based on CLSI breakpoint recommendations and published studies (Brown-Elliott & Woods, 2019). The reference strains that were used as part of this analysis included *M. kansasii* ATCC® 12478 for SGM and *M. peregrinum* ATCC® 700686 for RGM (Li et al., 2017). Fast-growing mycobacteria were seen on day 5 of incubation in comparison to the growth of the positive control well; observations were made on days 10 through 14. Re-testing the drug susceptibility test was advised if the growth of the positive control well did not improve by day 21 after incubation.

GenoType MTBDRplus V.2.0 assay on NTMs. The GenoType MTBDRplus V.2.0 assay was performed according to the manufacturer's protocol (Hain Lifescience GmbH. GenoTypeMTBDRplus, version 2.0 product insert. & Nehren, Germany). The test is based on DNA strip technology and has three steps: DNA extraction, multiplex PCR amplification, and reverse hybridization.

Ethical considerations. Ethical approval for this study was obtained from Masinde Muliro University of Science and Technology Institutional Ethical Review Committee (Protocol: MMUST/IERC/097/2022. The National Commission for Science, Technology, and Innovation (NACOSTI) also gave permission to carry out the study through permit number NACOSTI/P/23/22686. Written informed consent was obtained from each participant before enrolment. All HIV-1 infected ART-naive, TB and NTM infected study participants were referred for further treatment.

III. RESULTS

***Anthropometric and demographic characteristics*.** Anthropometric and demographic data are summarized in table 1 below. A total of 167 participants were purposively recruited to the study. They presented with TB-like symptoms including chronic productive cough lasting more than 2 weeks, loss of appetite, fever, fatigue, headache and night sweats satisfied the inclusion criteria were able to produce sputum. Majority of participants were male 124 (74.3%) compared to female 43 (25.7%). Out of the total 167 participants, 73 (43.1%) were HIV positive of which some were co-infected with NTM while 94 (56.9%) were HIV negative but some were infected with NTM. The median age was equal between the clinical groups. However, the body mass index (BMI) of the HIV positive clinical group was significantly (Median; 19.8, IQR; 8.5 kg/m^2, vs Median; 23.8, IQR; 7.3 kg/m^2, P=0.026) lower compared with the HIV negative. Similarly, the weight of the HIV positive clinical group was significantly (Median; 60.4, IQR; 19.3 Kg, vs Median; 68.1, IQR; 24.9 Kg, P=0.046) lower compared with the HIV negative. Consistently, there were higher rates of underweight in the HIV positive group 30 (41.1%) compared with the negative 12 (12.8%). Other variables including education level, religion, marital status as well as anthropometric measures such as height, waist circumference, hip circumference, mid upper arm circumference (MUAC) and bust were similar between the groups. Conversely, occupation was significantly different between the groups with those formally employed being mostly HIV negative 65 (69.1%) vs positive 38 (52.1%), P=0.024.

Table 1

Anthropometric and Demographic Characteristics

Characteristics	**HIV (-), n=94**	**HIV (+), n=73**	***P***
Gender			
Female	25 (26.6)	18 (24.7)	0.776[a]
Male	69 (73.4)	55 (75.3)	
Age	40.5 (24.0)	41.0 (17.0)	0.806[c]
Height	1.7 (0.1)	1.7 (0.1)	0.258[c]
Weight	68.1 (24.9)	60.4 (19.3)	**0.046[c]**
BMI	23.8 (7.3)	19.8 (8.5)	**0.026[c]**
Nutrition status			
Underweight	12 (12.8)	30 (41.1)	0.436[b]
Normal	46 (48.9)	14 (19.2)	
Overweight	36 (38.3	29 (39.7)	
WC	74.0 (9.3)	76.0 (9.5)	0.624[c]

HC	89.5 (9.0)	90.0 (6.3)	0.821[c]
MUAC	25.0 (4.0)	24.0 (2.0)	0.824[c]
BUST	84.5 (6.3)	85.0 (7.0)	0.631[c]
Chronic cough≥ 2weeks	71 (75.5)	63 (86.3)	0.786[a]
Purulent sputum	73 (77.7)	66 (90.4)	0.859[a]
Weight Loss	64 (68.1)	59 (80.8)	0.842[a]
Night Sweats	67 (71.3)	61 (83.6)	0.678[a]
Malaise	63 (67.0)	62 (84.9)	0.789[a]
Fatigue	68 (72.3)	59 (80.8)	0.895[a]
Hemoptysis	2 (2.1)	5 (6.8)	0.309[a]
Fever	54 (57.4)	48 (65.8)	0.467[a]

Data are presented as medians, interquartile range (IQR) or numbers (n) and percentages (%) of study subjects. HIV-1(+), human immunodeficiency virus type-1; BMI, body mass index; Normal; ≥18.5≤25.0 kg/m^2, Underweight <18.5 kg/m^2, Overweight ≥25.0 kg/m^2. MUAC, mid upper arm circumference, WC, waist circumference, HC, hips circumference, BMI; body mass index; *P*, [a]Fisher's exact tests; [b]Chi-square test and [c]Mann Whitney U test for continuous data. Bolded are significant *P*-values.

NTM species were isolated from sputum samples by cultural characteristics. Table 2 below shows the various NTMs identified. Out of 167 samples, 59 samples that grew in PNB- LJ medium were considered as NTM since, MTBC growth was inhibited by the presence of PNB in the media. The GenoType Mycobacterium CM/AS was used to speciate isolated NTM from liquid (BACTEC MGIT 960, Becton Dickinson, USA) or LJ-PNB culture media. the most common NTM identified were M. *intracellularae* 24/59 (40.7%), followed by *M. fortuitum* 15/59 (25.4%) and *M. avium* 7/59 (11.9%) respectively. Other species identified were, *M. kansasii* 7/59 (11.9%), *M. gordanae* 2/59 (3.4%). The species that had one isolate each were: *M. simiae, M. abscessus, M. scrofulacaeum* and *M. lentiflavum*.

Table 2

NTM Species Isolated from Sputum in HIV +/- Participants

NTM culture positive	**n (%)**
M. intracellularae	24 (40.7)
M. fortuitum	15 (25.4)
M. avium	7 (11.9)
M. kansasii	7 (11.9)
M. gordanae	2 (3.4)
M. simiae	1 (1.7)
M. abscessus	1 (1.7)
M. scrofulaceum	1 (1.7)
M. lentiflavum	1 (1.7)
Total	59 (100)

Data are presented as numbers (n) and percentages (%) of non-tuberculous mycobacterium

Summary of minimum inhibition concentrations (MICs). Figure 1 shows the percentage resistance of non-tuberculous mycobacterium (NTM) to the chosen antimycobacterial agents of HIV-negative and HIV-positive participants. Resistance patterns were also unique with the antibiotics tested. The proportion of resistance to isoniazid was based on high rates in both groups, and there was a higher percentage resistance in the isolates of HIV-negative participants (92.3) in comparison with the HIV-positive participants (88.0), and the difference between the two groups was statistically significant ($P < 0.0001$). In the case of rifampicin, the resistance was greater in the HIV-positive isolates (90.0 per cent) than the HIV-negative isolates (88.4 per cent) and the difference was significant ($P = 0.0004$). The overall resistance to streptomycin was moderate (59.6% in the case of HIV-negative isolates and 56.5% in the case of HIV-positive isolates) and the differentiation between the two was statistically significant ($P = 0.0245$). Ethambutol exhibited a less level of resistance as compared to the other drugs and resistance was 35.7 percent among HIV-negative isolates and 37.5 percent among HIV-positive isolates; this was not significantly different ($P = 0.0566$). Pyrazinamide had a consistently high resistance between the two groups with resistance in the isolates of HIV-negative being 96.0% and that of HIV-positive being 95.2% and no statistically significant difference between them ($P = 0.0788$). On the whole, the number implies a high resistance rate of NTM isolates to isoniazid and pyrazinamide, medium resistance rate to streptomycin, and relatively low resistance rate to ethambutol. The state of HIV was a significant determinant of resistance to isoniazid, rifampicin, and streptomycin and not to ethambutol or pyrazinamide.

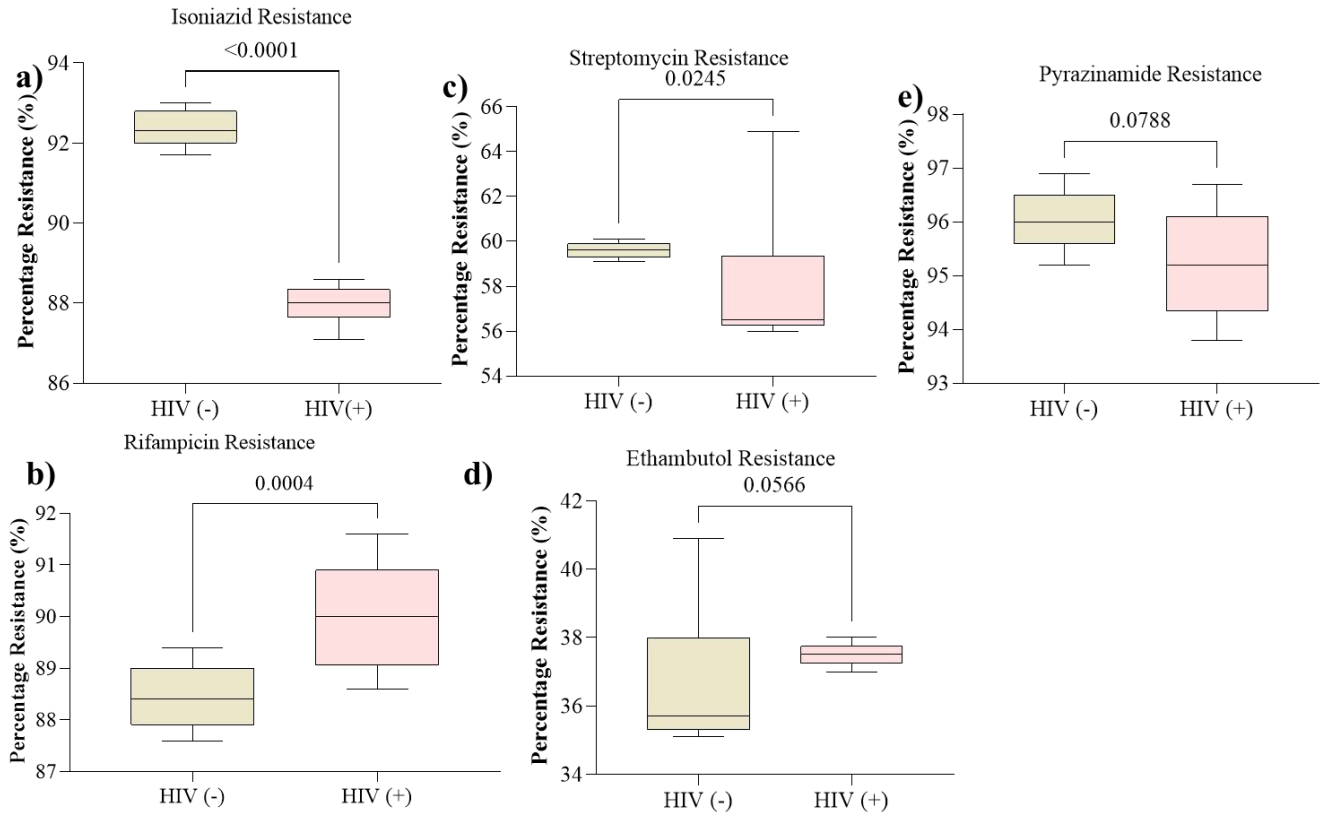

Figure 1
Minimum Inhibitory Concentration (Percentage Resistance per Antibiotic)

Figure 1: Comparison of percentage antimicrobial resistance among non-tuberculous mycobacteria isolates from HIV-negative and HIV-positive participants. Bars represent median percentage resistance to a) isoniazid, b) rifampicin, c) streptomycin, d) ethambutol, and e) pyrazinamide. Group comparisons were performed using the two-tailed exact Mann–Whitney U test. Resistance levels were consistently higher among isolates from HIV-positive individuals across all antibiotics tested.

Mutations Identified via Line Probe Assay on the NTM Isolates

Data on the single nucleotide polymorphisms among NTM infected individuals are summarized in table 3 below. The 3 missense mutations coding for resistance at the *rpoβ* locus were revealed to be D516V in HIV negative and 4 similar mutations were observed in HIV positive individuals. In addition, 2 H526Y and 1 H526D mutants were found to occur in the *rpoβ* locus among the HIV positive. In the *katG* gene, 3 and 7 individuals presented with mutations at codon 315 in HIV negative and positive individuals respectively. The single nucleotide mutations were specifically S315T. Only 2 mutations were described in the *inhA* locus C15T among the HIV positive participants.

Table 3
Single Nucleotide Polymorphism Identification by Line Probe Assays in NTM

Gene	**HIV (-)** (AA change)	**HIV (+)** (AA change)	Nucleotide change
rpoβ	D516V (3) H526Y (0) H526D (0) S531L (0)	D516V (4) H526Y (2) H526D (1) S531L (0)	GAC>GTC CAC>TAC TGG>TTG
katG	S315T (3)	S315T (7)	AGC>ACC
inhA	C15T (0)	C15T (2)	TCG>ACG

Data presented as codon mapping on LPA. Data is showing missense mutation leading to a change in specific locus of the genes

IV. DISCUSSION

The current study analyzed antimicrobial susceptibility patterns and characterized gene mutations associated with resistance to isoniazid and rifampicin in NTM isolates from HIV-1 infected patients. Generally, age and height were similar between clinical groups (HIV-1 negative and positive). Though, the participants were predominantly male. Similar previous studies have also reported a higher prevalence of TB-like symptoms and NTM infections among males (Dahl et al., 2022; Gopalaswamy et al., 2020). For example, a study conducted in Tanzania reported that males were more likely to present with TB symptoms and were more often diagnosed with TB and NTM infections (Maya et al., 2022). This trend may be attributed to gender specific behaviours, occupational exposures, and health seeking behaviours. Religion, educational level and marital status did not have any influence to any outcomes between the groups. Moreover, studies from different regions might report variations in the prevalence and demographic distribution of TB, NTM, and HIV infections. For example, studies in Asian countries have reported different gender distributions and occupational impacts compared to African studies (Dao et al., 2013). The results indicate that HIV positive participants with NTM infection had significantly lower BMI and weight compared to HIV-negative participants with NTM infection. The association between HIV infection and lower BMI and weight is well documented in the literature (Hill et al., 2024; Malvy et al., 2001). Studies in sub-Saharan Africa have consistently shown that HIV-infected individuals are more likely to experience malnutrition and lower BMI due to the increased metabolic demands of the infection, opportunistic infections, and reduced nutrient intake (Alebel et al., 2022; Fuseini et al., 2021; Martinez et al., 2016; Mpaka-Mbatha et al., 2022). Other anthropometric measures like height, waist/hip circumference, MUAC and bust were similar between the groups. Occupation differed, with more of the HIV-negative group formally employed. This association has been shown in other research as well. Formal employment often correlates with better socio-economic status, which in turn is associated with better access to healthcare and preventive measures (McMaughan et al., 2020). Studies in Southern Africa found that unemployment and informal employment were more common among HIV-positive individuals, potentially due to the physical and social impacts of the disease (Bor et al., 2012; Thomas et al., 2019). However, the role of socioeconomic factors such as employment status may differ in urban versus rural settings, influencing the generalizability of findings across different populations.

M. intracellulare was the most prevalent, followed by *M. avium*. Similar studies have frequently reported *M. intracellulare* and *M. avium* as the predominant NTM species. A study in the United States found *M. avium* complex (MAC), which includes both *M. avium* and *M. intracellulare*, to be the most common cause of NTM lung disease (Adjemian et al., 2012; Mullen et al., 2024). The high prevalence of these species in both studies underscores their significant role in NTM infections worldwide. *M. fortuitum* was the second most common species. *M. fortuitum*, a rapidly growing mycobacterium, is often reported in studies from various regions. For instance, a study in Mexico identified *M. fortuitum* as a common NTM species, particularly in patients with skin and soft tissue infections (Lopez-Luis et al., 2020). Its relatively high prevalence in this study aligns with findings from other parts of the world, indicating its widespread presence. *M. kansasii* accounted for 11.9% of NTM isolates. The prevalence of *M. kansasii* varies by region. In certain studies, from Europe and North America, *M. kansasii* is frequently associated with pulmonary infections (Narimisa et al., 2024). For example, a study in the UK reported *M. kansasii* as a significant cause of NTM lung disease, particularly among immunocompromised patients. The similar prevalence in this study highlights the global significance of *M. kansasii*. *M. gordonae* (3.4%), with single isolates of *M. simiae*, *M. abscessus*, *M. scrofulaceum*, and *M. lentiflavum*. The prevalence of these less common NTMs can vary widely. *M. gordonae*, often considered a contaminant, has been occasionally isolated in clinical settings. *M. abscessus* is notable for its pathogenic potential, particularly in cystic fibrosis patients, as reported in studies from the US and Europe (Degiacomi et al., 2019; Recchia et al., 2023). *M. simiae*, *M. scrofulaceum*, and *M. lentiflavum* are infrequently reported but have been documented in diverse geographic locations, underscoring the heterogeneity of NTM species distributions. The distribution of NTM species can differ significantly based on geographic location. For instance, MAC is more prevalent in North America and Europe, while rapidly growing mycobacteria like *M. fortuitum* are more frequently reported in tropical and subtropical regions (Delghandi et al., 2020; Ochayo et al., 2023). The type of clinical sample and patient population can influence the prevalence of specific NTM species (Winthrop et al., 2020). Studies focusing on respiratory samples often report higher rates of MAC, while studies involving skin and soft tissue infections may see a higher prevalence of rapidly growing mycobacteria like *M. fortuitum* and *M. abscessus*.

This study shows significant differences in resistance in terms of minimum inhibitory concentration-derived resistance between non-tuberculous mycobacteria (NTM) isolates, with resistance patterns depending on antibiotic and, in a few cases, on HIV status. In general, the high resistance to isoniazid/pyrazinamide, moderate resistance to streptomycin, and relatively low resistance to ethambutol were all in agreement with the known NTM resistance patterns (Ahmed et al., 2013; Calcagno et al., 2024). The well-reported intrinsic resistance of most NTMs is reflected in the high resistance of both groups to isoniazid and is caused by the lack of drug activation and the reduced cell wall permeability of mycobacterium. In spite of the observed differences between HIV-negative and HIV-positive groups,

the evenly high resistance proves the low clinical merit of isoniazid in NTM infections. Likewise, pyrazinamide had nearly universal resistance regardless of HIV status, which agrees with the lack of functional pyrazinamidase activity in NTMs, and the reason why it should not be included in the NTM treatment regimen. The resistance to streptomycin was moderate and varied greatly by HIV status. This variation is probably due to species makeup and previous exposure to antimicrobials, and implies lower accuracy of streptomycin in the treatment of NTM. On the contrary, the lowest levels of resistance were observed with ethambutol where there was no statistically significant difference in the levels of resistance across groups justifying its inclusion in combination therapy against specific NTM species but with susceptibility-based usage. The effects of the HIV status on the resistance against certain agents identified could be associated with the immunosuppression related to the bacterial persistence and selection (Miotto et al., 2018; Ogwang et al., 2021; Rockwood et al., 2015). These results emphasize the poor efficacy of conventional anti-tuberculosis agents in tackling NTMs and the need to identify individual species and perform regular susceptibility testing to inform the proper therapy, especially in resource-constrained environments.

Overall, these findings highlight the complexity of treating NTM infections, especially in HIV-positive individuals, and underscore the importance of tailored treatment strategies based on drug sensitivity and resistance profiles. The mutations identified in the *rpoβ* and *katG* genes, along with those in the *inhA* reflect patterns of drug resistance often observed in MTB strains, particularly in the context of HIV co-infection. The *rpoβ* gene, encoding the β subunit of RNA polymerase, is commonly associated with rifampicin resistance when mutated. The D516V, H526Y, and H526D mutations are well-documented in the literature. Studies such as those by Prammananan et al. (2008) and Jing et al (2017) confirm that these mutations are prevalent in rifampicin-resistant TB strains (Jing et al., 2017; Prammananan et al., 2008). However, few studies have documented these mutations in NTMs especially in HIV-1 infected patients. Research by Singh et al. (2023) and Gupta et al. (2011) indicates that rifampicin resistance is more frequently observed in HIV-positive TB patients (Gupta et al., 2011; Singh et al., 2023). This correlates with the higher number of *rpoβ* mutations observed in the HIV-positive group in the present study. This trend may be due to the higher bacterial load and faster progression of TB in immunocompromised individuals, which can lead to a higher mutation rate and drug resistance. In NTMs, *rpoβ* mutations also confer rifampicin resistance, but the specific mutations and their prevalence can vary. For instance, mutations in NTMs like *M. kansasii* may involve different codons. Contrastingly, Brown-Elliott et al. (2012) showed that NTMs may harbor unique *rpoβ* mutations not commonly seen in M. tuberculosis (Brown-Elliott et al., 2012).

The S315T mutation in the *katG* gene, responsible for encoding the catalase-peroxidase enzyme, is one of the most common mutations associated with isoniazid resistance. Numerous studies, such as those by Mokrousov et al. (2002) and Piatek et al. (2000), report high frequencies of the S315T mutation in both HIV-positive and HIV-negative TB patients (Mokrousov et al., 2002; Piatek et al., 2000). The higher occurrence of the S315T mutation in HIV-positive individuals in the current study aligns with findings from other research. For instance, studies by Narayanan et al. (2002) and Gandhi et al. (2006) have shown a significant association between HIV infection and higher rates of isoniazid resistance (Gandhi et al., 2006; Narayanan et al., 2002). This may be attributed to the increased use of isoniazid preventive therapy in HIV-positive populations, which can select for resistant strains. In NTMs, the *katG* gene's role in isoniazid resistance is less prominent because NTMs are generally less susceptible to isoniazid. For example, *M. avium* and *M. abscessus* are intrinsically resistant to isoniazid, rendering *katG* mutations less relevant. However, studies such as those by Griffith et al. (2007) have identified *katG* mutations in some NTM species, indicating that resistance mechanisms can vary (Griffith et al., 2007).

The C15T mutation in the promoter region of the *inhA* gene, which encodes the enoyl-ACP reductase enzyme, leads to overexpression of the enzyme and confers low-level isoniazid resistance. Studies such as those by Isakova et al. (2018) and Spies et al. (2008) have identified this mutation in both HIV-positive and HIV-negative TB patients, but it appears less frequently compared to *katG* mutations (Isakova et al., 2018; Spies et al., 2008). The presence of *inhA* mutations specifically in HIV-positive individuals, as observed in the current study, might suggest a unique resistance pattern driven by the interplay between HIV infection and NTM treatment regimens. This aligns with findings from studies like those by Seifert et al. (2015) and Naidoo et al. (2015), which highlight different resistance profiles in HIV-positive populations (Naidoo et al., 2015; Seifert et al., 2015). The *inhA* locus mutations in NTMs may contribute to resistance against other drugs like ethionamide. In *M. tuberculosis*, *inhA* mutations confer resistance to isoniazid and ethionamide. In NTMs, however, the clinical significance of *inhA* mutations is less clear. Research by Nasiri et al. (2017) indicates that NTMs may have different regulatory mechanisms affecting drug resistance (Nasiri et al., 2017). The immune status of HIV-positive individuals can influence the resistance profiles of both *M. tuberculosis* and NTMs. A previous study for instance Agizew et al. (2020) highlights that HIV-positive patients often present with multi-drug resistant NTMs, complicating treatment regimens. In general, HIV-positive individuals are at higher risk of NTM infections due to immunosuppression (Agizew et al., 2020). The presence of NTMs can complicate the treatment of TB, as NTMs can be resistant to standard TB medications.

Overall, understanding the specific resistance mechanisms in NTMs is essential for designing effective treatment strategies, especially in HIV-positive patients who might be co-infected with M. tuberculosis and NTMs.

V. CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study provides critical insights into the antimicrobial resistance patterns and genetic mutations among non-tuberculous mycobacterium isolates from HIV-1 patients in Western Kenya, revealing that NTM infections pose a significant and often underestimated clinical challenge in immunocompromised populations. The predominance of *M. intracellulare* and *M. fortuitum* among the 59 NTM isolates, coupled with alarmingly high resistance rates to first-line anti-tuberculous drugs—particularly isoniazid (88–92%), rifampicin (88–90%), and pyrazinamide (95–96%)—underscores the inadequacy of standard TB treatment regimens for NTM infections. The identification of clinically relevant mutations in the rpoβ (D516V, H526Y, H526D), katG (S315T), and inhA (C15T) genes, with higher mutation frequencies observed in HIV-positive individuals, confirms that drug resistance mechanisms in NTMs share genetic similarities with *M. tuberculosis* while also exhibiting species-specific patterns that complicate diagnosis and treatment. The significant association between HIV positivity and lower BMI, reduced weight, and higher rates of undernutrition further highlights the compounded vulnerability of immunocompromised patients to both NTM infection and adverse treatment outcomes. Collectively, these findings demonstrate that NTM infections in HIV-endemic settings represent a distinct clinical entity that requires specialized diagnostic approaches, individualized treatment strategies, and continuous antimicrobial resistance surveillance to improve patient outcomes and mitigate the spread of drug-resistant mycobacterial strains.

5.2 Recommendations

Based on the study findings, we recommend the implementation of routine NTM species identification and antimicrobial susceptibility testing for all HIV-positive patients presenting with presumptive pulmonary tuberculosis who test negative for MTB complex, as this would prevent inappropriate treatment with standard anti-TB drugs and enable targeted therapy based on species-specific resistance profiles. Healthcare facilities in resource-limited settings should integrate molecular diagnostic tools such as line probe assays into their laboratory algorithms to facilitate rapid detection of both NTM species and resistance-conferring mutations in the rpoβ, katG, and inhA genes, thereby guiding evidence-based treatment decisions and reducing morbidity and mortality associated with misdiagnosis. The national tuberculosis and HIV control programs in Kenya should develop and disseminate clinical guidelines specifically addressing the management of NTM infections in HIV-positive patients, emphasizing the limited utility of isoniazid and pyrazinamide in NTM treatment while identifying alternative drug regimens based on susceptibility patterns observed in the region. Furthermore, we recommend establishing sentinel surveillance systems for antimicrobial resistance in NTM across Kenyan counties to monitor emerging resistance trends, track the spread of multidrug-resistant strains, and inform national treatment policies. Given the significant association between HIV infection, malnutrition, and NTM disease, comprehensive care packages that integrate nutritional support, antiretroviral therapy optimization, and regular clinical monitoring should be provided to HIV-positive patients diagnosed with NTM infections to address the interrelated factors contributing to poor treatment outcomes. Finally, we advocate for future research focusing on the clinical outcomes of NTM-infected HIV patients receiving tailored antimicrobial therapy, the mechanisms of horizontal gene transfer contributing to AMR spread among environmental and clinical NTM isolates, and the development of novel diagnostic tools and therapeutic agents specifically designed to address the unique resistance profiles of NTMs prevalent in Sub-Saharan Africa.

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