International Journal of Innovation Scientific Research and Review
Vol. 04, Issue, 08, pp.3183-3189, August 2022
Available online at http://www.journalijisr.com
SJIF Impact Factor 4.95

IJISRR

ISSN: 2582-6131

Research Article

COMPARISON OF Geno Type MTBDR*plus* WITH BACTEC MGIT 960 FOR ISONIAZID AND RIFAMPICIN *Mycobacterium tuberculosis* DRUG RESISTANCE MONITORING IN HIV PREDOMINANT REGION OF WESTERN KENYA

[1,2] * Fredrick Ogumbo, [1]Ronald Odero, [1]Ben Odhiambo, [1]Patrick Emojong, [1]Arthur Odoyo, [1]Albert Okumu, [2]James Nonoh, [2]Benard Guya, [1]Steve Wandiga

[1]Kenya Medical Research Institute, Centre for Global Health Research, Kenya.
[2]Maseno University, Department of Biomedical Science and Technology, Kenya.

Received 21st June 2022; Accepted 22th July 2022; Published online 31st August 2022

ABSTRACT

Background: Globally, tuberculosis drug resistance is one of the leading poor clinical outcomes of treatment. Early detection of drug resistant strains is one of the tools that has been used in drug resistance monitoring and molecular line probe assays have been alternatives to phenotypic methods. Although there is evidence of the accuracy of the line probe assay for tuberculosis drug resistance monitoring, the prevalence of drug resistant tuberculosis varies regionally and this variation in prevalence has a significant impact on the predictive value of the line probe assay. As such this study aimed to evaluate the performance of line probe assay for detection of isoniazid and rifampicin *Mycobacterium tuberculosis* drug resistance in Human Immunodeficiency Virus endemic region of Western Kenya. **Methods:** In this prospective cross sectional study, the investigation evaluated the performance of GenoType MTBDR*plus* in detection of drug resistance. Using BACTEC MGIT 960 as the gold standard, sensitivity, specificity, positive predictive value and negative predictive value of the two assays were compared and the pattern of mutations associated with resistance to rifampicin and isoniazid analyzed. **Results:** GenoType MTBDR*plus* correctly identified isoniazid resistance 9/11 and rifampicin 7/10 and a statistical significant relationship with BACTEC MGIT 960 for detection of isoniazid and rifampicin resistance, $p<0.05$, area under the curve 0.909 and 0.85 respectively indicating an excellent diagnostic accuracy. **Conclusion:** Geno Type MTBDR plus assay was found to be consistent method for direct detection of resistance to isoniazid, rifampicin and multidrug resistance tuberculosis directly from sputum samples in HIV endemic regions of Western Kenya.

Keywords: Isoniazid resistance, rifampicin resistance, multidrug resistance tuberculosis.

INTRODUCTION

Background

Tuberculosis is a communicable disease caused by the tubercle bacillus and the emergence and spread of drug resistant strains is a worldwide concern. Globally, the prevalence of drug-resistant tuberculosis (DR-TB) has increased substantially within the past 20 years making tuberculosis (TB) the leading single cause of death among persons living with human immunodeficiency virus (HIV) infection and accounting for about 40% of deaths among this population (1, 2). Tuberculosis has been associated with poor clinical outcomes especially in low income settings and is usually the primary indicator of HIV infection (3). World Health Organization reported that there was an estimated 9.0 million new cases of tuberculosis globally in 2019 (4), half of these incidences (56%) were within the South-East Asia and Western Pacific regions, while 29% in the African region (5). Additionally, WHO estimated that, 8.6% cases with active TB were also co infected with HIV in 2018 (6), while rifampicin-resistant (RR) or multidrug-resistant (MDR) TB occurred among 3.6% and 18% of new and retreatment TB cases respectively (5.6% among all cases) (7). Inappropriate use of antibiotics in treatment of drug susceptible patients, sub-optimal treatment regimens and failure to finish treatment in drug susceptible patients have been postulated as some of the causes of drug resistance (8). This has therefore resulted in high treatment failures and death rates exuberated by complexities in diagnosis and treatment (9). Kenya is among the 14 countries globally that are in the lists of high burden countries for TB, TB/HIV and MDR-TB and the fifth highest burden in Africa (4). The estimated incident for TB within the country is 348/100,000 population, translating to about 169,000 TB cases occurring annually, the death rate (excluding HIV+TB) is 60/100,000 population (10). According to World Health Organization, 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 has its greatest toll in the age bracket of 15 to 44 years and therefore the major factor liable for the massive TB disease burden in Kenya and contributor to 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 from 2015 to 2017 found that, out of a total of 132 tuberculosis patients screened, just two patients showed resistance associated with first- and second-line TB drugs (13) and additionally of these two patients, one developed resistance to Isoniazid, while the other depicted a case of MDR and there was no case of both INH and RIF (13). Out of a total 132 patients screened for the resistance to second-line drugs, one cross-resistance was detected for Aminoglycosides and Fluoroquinolones antibiotics (13). On comparing susceptibility between first-line and second-line drug-sensitivity, it had been noted that the MDR-TB case had an extra second-line drug resistance while the mono-resistant case had no additional second-line drug resistance (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). Drug resistant TB strains are mainly caused by inadequate treatment of TB patients and the emergence

***Corresponding Author: Fredrick Ogumbo,**
1Kenya Medical Research Institute, Centre for Global Health Research, Kenya.
2Maseno University, Department of Biomedical Science and Technology, Kenya

and spread of these strains is an obstacle to the control and management of tuberculosis hindering the World Health Organization's goal of eliminating the disease by 2050 (4). Proper management of MDR-TB relies on early detection of drug resistant strains and recently phenotypic and genotypic diagnostic methods have been deployed to rapidly identify strains that confers resistance to antituberculosis agents. In phenotypic drug susceptibility testing, *Mycobacterium tuberculosis* is detected and/or characterized with regard to its drug susceptibility using direct or indirect observations of cellular growth and/or bacterial metabolism from chosen media(3).Molecular Line probe assays are nucleic acid amplification technologies that have been approved by WHO for rapid detection of tuberculosis drug resistance using culture isolates or as direct testing of acid fast bacilli from both smear positive and smear negative sputum specimens (15). However, the prevalence of drug resistant tuberculosis varies regionally and this variation in prevalence has a significant impact on the predictive value of the line probe assay. This study thus aimed to evaluate the performance of Line Probe Assay in comparison to phenotypic drug susceptibility testing for detection of *Mycobacterium tuberculosis* drug resistance.

MATERIALS AND METHODS

Study Design and setting

Hospital and laboratory based descriptive cross sectional study design was used to collect sputum samples from tuberculosis patients attending TB clinics and hospital facilities within Kisumu County, Western Kenya. The study was conducted between November 2020 and October 2021, to compare the performance of Molecular line probe assay (GenoType® MTBDR*plus*) with phenotypic (BACTEC™ MGIT™ 960 system) (Becton Dickinson, Franklin Lakes, NJ, USA) in detection of *Mycobacterium tuberculosis* drug resistance among new and previously treated pulmonary tuberculosis cases from Kisumu County, Kenya.

Sampling technique

This study employed 100 percent sampling of all tuberculosis suspected patients attending various health facilities within Kisumu County. Saturated sampling was preferred in this study because TB Clinics and Hospital facilities within Kisumu County were quite few. The study sample included clinically suspected tuberculosis patients attending various health facilities in Kisumu County who meet the inclusion criteria and provided informed consent.

Sampling technique

This study employed saturated sampling of all tuberculosis suspected patients attending various health facilities in Kisumu County. All eligible patients presenting to health centre in the study area within the defined study period were enrolled and this method was preferred because TB Clinics and Hospital facilities within Kisumu County was quite few. The study sample frame included all clinically suspected tuberculosis patients attending various health facilities in Kisumu County who met the inclusion criteria and provided informed consent.

Data Collection

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

Sample collection and Processing

Patient who met the minimum inclusion criteria were recruited into the study. They were then given sputum cups by the clinician or laboratory personnel within the recruiting facility so as to have their sputum samples taken. A pipette drop from the sample was taken to bacteriology confirm the sample for acid fast bacilli at the clinic and an aliquot of the sample was then parked in screw cups with double biohazard bags inside a cooler box and transported to Kenya Medical Research Institute (KEMRI) Microbiology reference laboratory in Kisian for further confirmatory staining, culturing and Molecular drug resistance testing. Local specimen shipment was carried out in line with regulation provided by the International air transport Association (http://www.iata.org/ ads/issa/htm). At KEMRI Microbiology reference laboratory, sputum samples together with the lab request form were received from health facilities within the County and checked for completeness in filling the laboratory request form, correct sample tube labeling and leakage. Those meeting the acceptance criteria were assigned laboratory study number and refrigerated at +4°C awaiting processing**.**

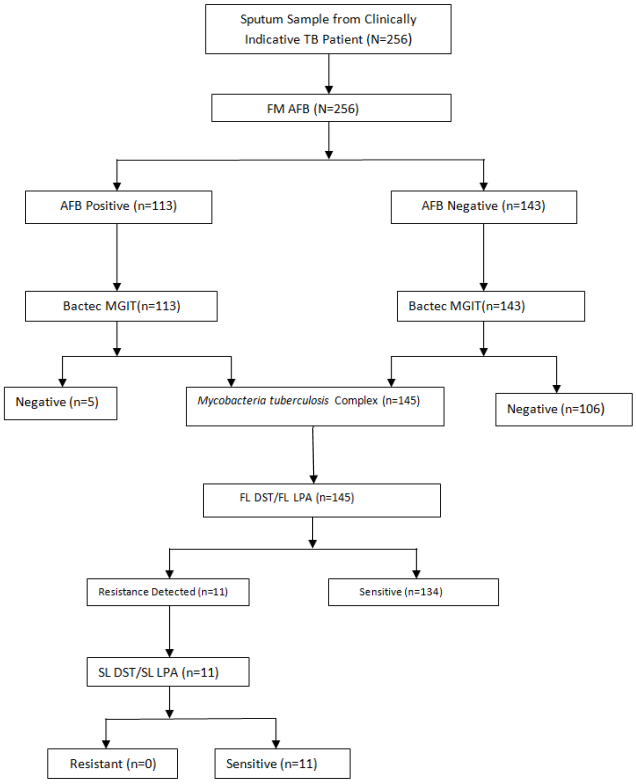

Figure 1: Flow Chart of Sample processing

Decontamination and Ziehl-Neelsen (ZN) smear microscopy

Decontaminated of sputum specimen was done using the N-acetyl-l-cysteine-sodium citrate-NaOH (NALC-NaOH) method [18]. Samples were then decanted following centrifugation at 3000 g for 15 minutes, and the pellets re-suspended to make 3 ml using phosphate solution. Four aliquots of 1.0 ml were made from the stock sample,1 aliquot was used for florescent microscopy, another for phenotypic drug susceptibility testing, Line Probe Assay and the last other remaining stored at − 80 °C as a retesting sample. Staining and microscopy was done as follows; Carbol fuchsin was prepared to flood heat-fixed sputum sample smears. The flooded slide was heated to steam with a flame and let air dry for 10 min, then washed with water and decolorized using 3% acid alcohol. After which, the smear was flooded with malachite green and left to stain for 2 min. This stain was then washed with water and smear air dried and later observed

microscopically using X100 oil immersion objective (16). Microscopy was done for all the 256 Sputum samples.

Phenotypic testing

Phenotypic drug susceptibility testing of *Mycobacterium tuberculosis* isolates to the first line drugs was done using BD BACTEC™ MGIT™ 960 system (Becton Dickinson, Franklin Lakes, NJ, USA) within 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 15.0 ml MGIT growth supplement provided. A micropipette was then aspirated to transfer, 0.8 ml of the mixture to each MGIT tube to be inoculated with specimen including both negative and positive controls. Making use of 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 3 times to allow proper constitution of the mixture. The MGIT tubes were then inserted into the BACTEC machine after scanning each tube [15]. The instrument maintained a temperature of 37 °C + or - 1 °C, which was the optimum growth temperature for M. tuberculosis. Mycobacteria Growth Indicator tubes were then incubated until flagged positive by the instrument as for the negative tubes, they were flagged after a maximum of 6 weeks when no growth occurred. The MGIT tubes that were positively flagged were then removed and scanned.

Molecular Line Probe Assay

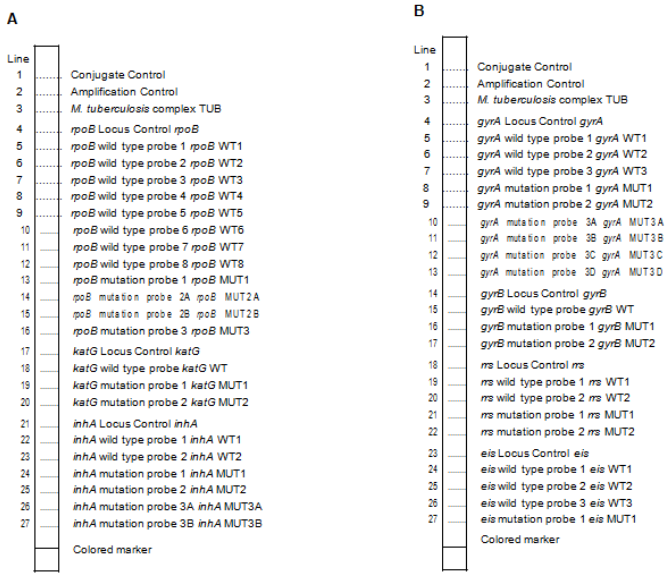

Figure 2: Configuration of GenoType MTBDRplus V2 (a) and GenoType MTBDRsl V2 (b) strips

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

Interpretation

Each strip of Line Probe assay had 27 reaction zones and these including six controls bands conjugate band, *M. tuberculosis* complex, amplification, rpoB, inhA and katG, eight rpoB wild type (WT1–WT8) and 4 mutant probes *(rpoB MUT D516V, rpoB MUT H526Y*, *rpoB MUTS531 L and rpoB MUT H526D),* one katG wild type, two mutant and two inhA wild type and four mutant probes. Either missing wild type band or the presence of mutant band was taken as a symbol of a resistant strain. To provide a consistent result, all six expected control bands appeared correctly. Otherwise, the result was considered invalid.

Statistical Methods

SPSS v23 (SPSS Software | IBM) was used for data analysis and it merged both the clinical and the laboratory databases before analysis. Participants were classified as new, previously treated tuberculosis cases and characteristic of study participant were compared between new tuberculosis cases and previously treated cases using Chi-square test for categorical variables.Diagnostic accuracy was done according to STARD "Standards for Reporting Diagnostic accuracy studies" Using BACTEC™ MGIT™ 960 as gold standard, diagnostic accuracy of GenoType® MTBDR*plus* for detection of first line drug resistance was assessed. Chi square was therefore used to determine the accuracy of the GenoType® MTBDR*plus* assay to detect rifampicin and Isoniazid drug resistance. Cross tabulation was additionally useful in assessing the specificity verses sensitivity and positive predictive value verses negative predictive value with 95% confidence intervals (CI) of the MTBDR*plus* assay for the MDR-TB detection, rifampicin and isoniazid resistance. Sensitivity was described as the proportion of isolates correctly determined as resistant by the GenoType® MTBDR*plus* assay compared with BACTEC™ MGIT™ 960. The definition of specificity was described as the proportion of tuberculosis isolates that were correctly determined as susceptible by the GenoType® MTBDR*plus* assay compared with BACTEC™ MGIT™ 960. Positive predictive value was defined as the proportion of resistant isolates determined by the BACTEC™ MGIT™ 960 among isolates determined as resistant by the GenoType® MTBDR*plus* assay. Negative predictive value was defined as proportion of susceptible isolates determined by the BACTEC™ MGIT™ 960 system among isolates determined as susceptible by the GenoType® MTBDR*plus* assay. The degree of agreement between BACTEC™ MGIT™ 960 system and GenoType® MTBDR*plus* assay was also assessed using area under the curve. Diagnostic accuracy was assessed by area under the curve as follows; area under the curve of 0.9 - 1.0: excellent, 0.8 - 0.9: perfect, 0.7 - 0.8: good, 0.6 to 0.7 as sufficient, 0.5 to 0.6 bad diagnostic tool while < 0.5 defined as test not useful.

Ethical Clearance

Ethical approval was done by Kenya Medical Research Institute, Scientific Ethical Review Unit (KEMRI/SERU/CGHR/002-02-330/4079) and National Commission for Science, Technology & Innovation (NACOSTI/P/21/10981). Written consent was obtained from study participants.

RESULTS

Socio-demographic Characteristics of the respondents

The study found out that, out of sample size of 256, 168(65.5%) were male while the remaining 88 (34.4%) were female. Ages under 18 years were 5(2%), while 18 years and above 251(98%). All the participants had a mean age of 40 years with a standard deviation of ± 12.9 and a range of 13 to 77 years.

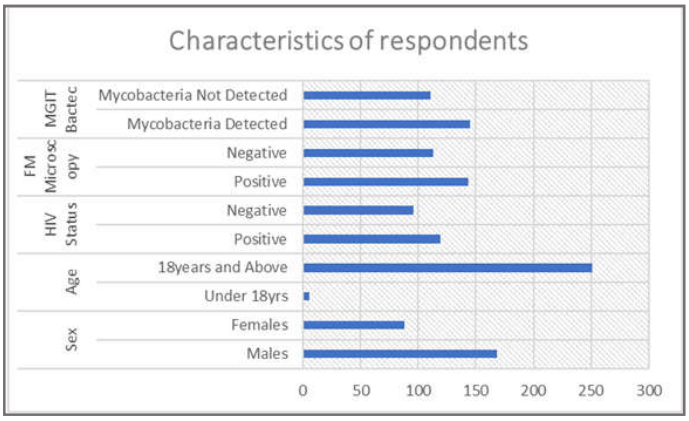

Figure 3: Socio demographic Characteristic of the study Population

Characteristic of TB Confirmed cases

Out of sample size of 256, 145 were mycobacteria confirmed cases and the male *Mycobacterium tuberculosis* confirmed cases were 112(66.7%), while female confirmed cases were 33(37.5%). Out of 145 mycobacteria confirmed cases on phenotypic testing 32 (22%) were from new TB cases and 113(78%) retreatment. Males confirmed cases were 112 (77.2%) while females were 33(22.8%). Ages under 18 years confirmed cases were 2(1.4%), while 18 years and above 143(98.6%).

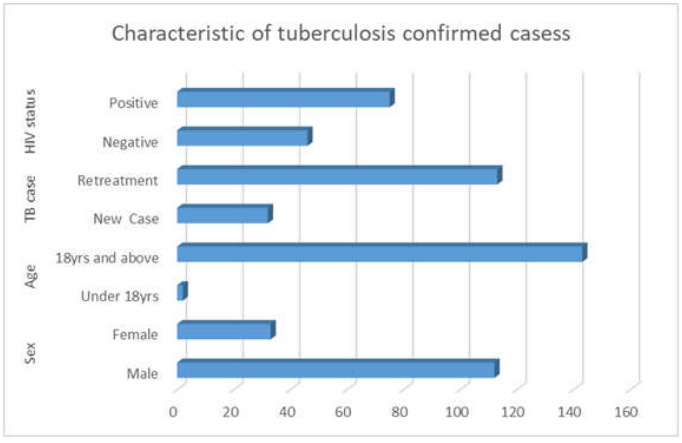

Figure 4: Graph of Characteristic of Tuberculosis confirmed cases

Sub County drug resistance profile

From a sample of 145 tuberculosis confirmed cases on phenotypic culture, rifampicin resistance was highest in Seme Sub-County 4 (2.8%) and isoniazid 3 (2.1%); followed by Kisumu Central rifampicin 3 (2.1%) and isoniazid 3 (2. 1%). No resistance to both isoniazid and rifampicin were experienced in Muhoroni and Nyakach Sub-counties. Out of the 4 (2.8%) MDR cases, 3 (2.1%) were from Seme, while 1 (0.7%) was from Kisumu Central Sub-county. All the 3 MDR cases from Seme were from the same facility (Body health Centre) and 1 case from Kisumu central was from Lumumba Health Centre.

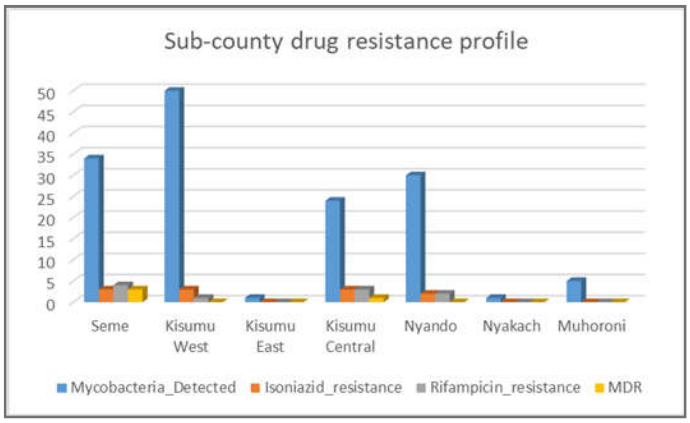

Figure 5: Sub County drug resistance profile

Drug resistance in TB cases

Out of one hundred and forty-five Tuberculosis confirmed cases, 11(7.6%) showed resistance detected for Phenotypic DST, of which was 2(6.3%) were in new TB case and 9(8.0%) in retreatment case. Chi square for Phenotypic DST Isoniazid resistance and TB cases was ($\chi 2$ =0.13, df=1, p =0.908), OR 1.096(0.279-5.240), 95 %CI. The study found out that, Phenotypic DST for rifampicin resistance was 10(6.9%), of which was 1(6.3%) was in new TB case and 9(8.0%) in retreatment case. Chi square for Phenotypic DST for rifampicin resistance and TB Cases was, ($\chi 2$ =0.602, df=1, p =0.438), OR 2.239(0.277-18.09), 95 %CI. LPA for isoniazid resistance detected was 9(8.0%) of which 2(6.2%) was in new TB case and 7(5.3%) in retreatment case. Chi square for LPA Isoniazid and TB cases was, ($\chi 2$ =0.043, df=1, p =0.836) OR 0.844(0.170-4.193), 95 %CI. Out of 145 samples Tuberculosis confirmed cases, LPA for rifampicin resistance detected was 7(4.8%) of which 1(3.1%) was in new TB case and 6(4.5%) in retreatment case. Chi square for LPA Rifampicin and TB cases was, ($\chi 2$ =0.126, df=1, p =0.723), OR 1.47(0.173-12.496), 95 %CI. GenoType MTBDRplus showed that isoniazid and rifampicin Monoresistance was 7(4.8%) and 6(4.1%) respectively while isoniazid and rifampicin MDR was 2(1.4%).

Table 1: Drug resistance in TB cases

	New TB Cases N (%) n=32	Retreatment N(%) n=113	Total Resistance N (%) n=145	P Value [95%CI]	OR[95%CI]
DST Isoniazid	2(6.3)	9(7.9)	11(7.6)	0.908	1.096(0.279-5.240)
DST Rifampicin	1(3.1)	9(7.9)	10(6.9)	0.438	2.239(0.277-18.09)
LPA Isoniazid	2(6.3)	7(6.2)	9(6.2)	0.836	0.844(0.170-4.193)
LPA Rifampicin	1(3.1)	6(5.3)	7(4.8)	0.723	1.47(0.173-12.496)

First line drug resistance conferring mutations

Out of one hundred and forty-five tuberculosis confirmed cases, phenotypic drug resistance showed 7 (4.8%) isoniazid monoresistance, 6 (4.1%) showed rifampicin monoresistance resistance while 4 (2.8%) and phenotypic MDR was 4 (2.8%). Out of the 4 (2.8%) MDR cases, 3 (2.1%) were from Seme, while 1 (0.7%) was from Kisumu Central Sub-county. All the 3 MDR cases from Seme were from the same facility (Body health Centre) and 1 case from Kisumu central was from Lumumba Health Centre.For Genotype MTBDR plus, 7 (4.8%) showed Isoniazid monoresistance, 5 (3.4%) rifampicin monoresistance, while molecular MDR was 2 (1. 4%). GenoType MTBDRplus showed that out of the Seven isoniazid resistance, 5 (3.4%) had mutations in the *katG MUT1* showing high level isoniazid resistance, while 2 (1.4%) *inhA MUT1* showing low level of isoniazid resistance. Mutations associated with rifampicin resistance was detected at probes *roB MUT2A* 4 (2.8%) *and roB MUT3* 1 (0. 7%). Molecular MDR sowed hereto resistance to isoniazid and rifampicin, 1 sample showed mutations in the *roB MUT3, katG MUT1* probes while the other deletions in the *roB WT7*, *katG WT*. Two MDR cases were from the same facility Body health Centre in 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 *roB 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: Percentages for phenotypic and molecular drug resistance

Percentages for First Line Drug resistance

		Resistance Detected Frequency (%) n =145	Resistance Not Detected Frequency (%) n=145
MGIT BACTEC	INH Monoresistance	7(4.8)	138(95.2)
	RIF Monoresistance	6(4.1)	139(94.9)
	MDR	4(2.8)	141(97.2)
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)

Validation of molecular Line probe assay using MGIT as gold standard

Out of a sample size of 256, phenotypic drug resistance testing showed 145 (56.6%) Mycobacteria detected while 111 (43.4%) showed mycobacteria not detected. Using Culture for MGIT as gold standard, First Line LPA for tuberculosis had sensitivity of 73.8% and a positivity of 100%. Positive predicative value of 100% and a negative predicative value of 74.5%. Chi square for MGIT BACTEC and LPA for detection of Tuberculosis detection, ($\chi 2$ =140.732, df=1, p =0.000), AUC=0.869,95% CI.

Table 3: Diagnostic performance of Genotype MTBDRplus assay in detecting resistance in clinical isolates of *Mycobacterium tuberculosis*

Performance of the Genotype MTBDRplus assay in detecting resistance in clinical isolates of *Mycobacterium tuberculosis*
Gold standard: BACTEC MGIT DST 960 System

		RIF n=145	INH n=145	MDR n=145
Test Assay MTBDR*plus*	Resistant	7	9	2
	Resistant Not Detected	138	136	133
	Sensitivity(95% CI)	70	81.8	98.2
	Specificity(95%CI)	100	100	100
	PPV (95% CI)	100	100	100
	NPV(95%CI)	97.8	98.5	99.4
	AUC(95%CI)	.850	909	869
	Asymptotic Sig.[b]	.000	.000	000000

a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

Molecular line probe assay for isoniazid resistance using Phenotypic isoniazid as gold standard

Out of a sample size of 256, MGIT BACTEC showed 145 (56.6%) Mycobacteria detected while 111 (43.4%) showed mycobacteria not detected. From sample of 145 *Mycobacteria tuberculosis* Complex, DST isoniazid resistance was 11 (7.6%) isolates, while no resistance detected was 134 (92.4%). LPA isoniazid resistance detected was 9 (6.2%) while No resistance detected was 136 (93. 8%).The sensitivity of Line prove assay for detection of Isoniazid resistance was 81.8% while the specificity was 100%. The positive predicative value of LPA was 100% while the Negative predictive value was 99.2%. Chi square for LPA and DST for Isoniazid resistance detection, ($\chi 2$ =207.759, df=1, p =0.000), AUC=0.909,95% CI.

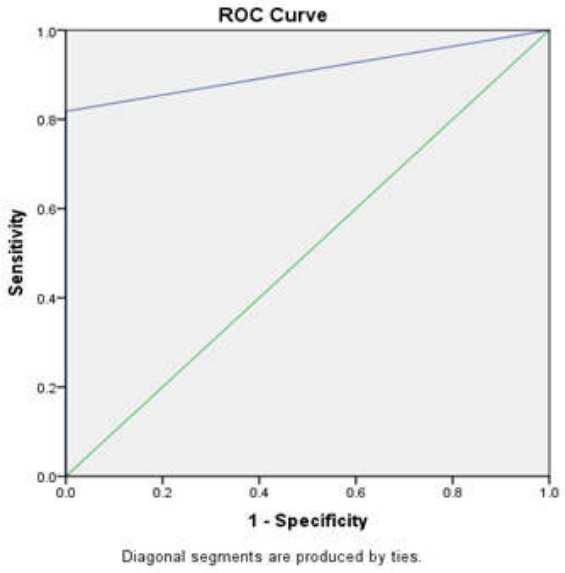

Figure 6: ROC of line probe assay for isoniazid resistance

Molecular line probe assay for rifampicin resistance using phenotypic rifampicin as gold standard

For culture DST, rifampicin resistant was 10 (6.9%), while resistance not detected was 135(93. 1%), out 145 that showed mycobacteria

detected on MGIT BACTEC. LPA rifampicin resistance was 7 (4.8%). The Sensitivity for LPA in detection of rifampicin resistance was 70% while specificity was 100%. The positive predicative value of LPA was 100% while the Negative predictive value was 98.8%. Chi square for LPA and DST for rifampicin resistance detection, ($\chi 2$ =177.041, df=1, p =0.000), 95% CI. Using DST Culture for rifampicin as gold standard, the ROC Curve for rifampicin resistance showed an Area under the curve of 0.85.

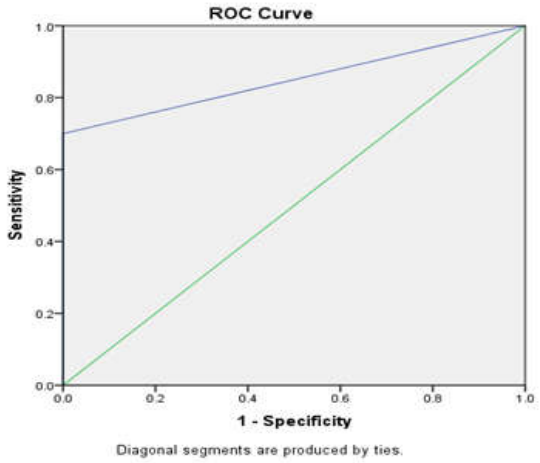

Figure 7: Receiver operating Characteristic curve of line probe assay for rifampicin resistance

DISCUSSION

A total of 256 sputum samples from tuberculosis clinical suspected cases from Kisumu County, Kenya for period of 12 months, November 2020 to October 2021 were included in the study. 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 cases 50 (34.5%), while Kisumu East and Nyakach Sub counties had 1 (0.7%) each. From 145 confirmed cases there were more males 112 (77.2%) compared to females 33 (22.8%), which is in agreement with the WHO report that relatively more males than females are exposed to tuberculosis which may be attributed to the difference between the two sex groups in biological, societal role and access to health facilities (4). Among tuberculosis confirmed cases, majority of participants were Ages 18 years and above 143 (98.6%), while the remaining 2 (1.4%), 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 current study is in good agreement with a study reporting that the 31–40 years age group was the most predominant group for isolation of DR-TB and the male population was at the highest risk (17).Findings from WHO shows that detection of Multidrug resistance and or rifampicin resistance requires bacteriological confirmation of tuberculosis and testing for drug resistance using Nucleic acid amplification technologies, and culture methods (18, 19). The current study reports the findings of the accuracy of the Genotype MTBDR*plus* assay for detection of drug resistance in new and previously treated tuberculosis patients in Kisumu County. Using Culture for MGIT as a reference standard for *Mycobacteria tuberculosis* complex, first line-line probe assay for tuberculosis had a sensitivity of 73.8% and a positivity of 100%. Positive predicative value of 100% and a negative predicative value of 74.5%. The study found out that there was a strong evidence of relationship between first line-line probe assay and Culture MGIT and for detection résistance, p<0.05. The area under the curve was 0.869 showing a higher discriminating power of the test hence a very good diagnostic accuracy. A number of studies show that GenoType MTBDR*plus* VER2.0 shows good accuracy for the detection of MDR isolates in smear-positive specimens (sensitivity between 83.3 and 96.4%, and specificity between 98.6 and 100%) (1, 2). This consistent with the current studies which showed a statistical significant relationship between molecular Line probe assay and Phenotypic MGIT culture for detection of isoniazid resistance<0.05. The sensitivity of Line prove assay for detection of isoniazid resistance was 81.8% while the specificity was 100%. The positive predicative value of LPA was 100% while the Negative predictive value was 98.5%. The area under the curve was 0.909 showing a better test in discriminating drug resistance from non-resistance hence presenting an excellent diagnostic effectiveness.The study found astrong evidence of of relationship between moleculat Line probe assay and Phenotypic MGIT culture for detection of rifampicin resistance,p<0.05. The Sensitivity for LPA in detection of rifampicin resistance was 70% while specificity was 100%. The positive predicative value of LPA was 100% while the Negative predictive value was 97.8%. Using DST Culture for rifampicin as gold standard, the area under the curve was 0.85 showing a better test in discriminating drug resistance from non-resistance hence presenting a very good diagnostic accuracy. The high specificity of the MTBDR*plus* assay in detecting INH resistance, RIF resistance and MDR MTBC isolates is consistent with the specificity in a number of previous studies (20-22). The proportion of MDR was high (1.8%) and all in retreatment cases. However, a sensitivity analysis indicates that the high proportion of MDR-TB patients in our study does not affect the positive predictive value of the Genotype MTBDR*plus* in diagnosing MDR-TB due to the high specificity value (100%) obtained. In this study, a number of RIF-resistant isolates failed to hybridize with one or two of the wild type (WT) probes and did not hybridize with any of the probes representing known mutations. These results could be an indication of a new previously unreported mutation. The WT probes with no hybridization were mostly *WT2, WT3, WT4* and *WT8*.According to Abanda *etal.,* this type of result could be likely linked to the failure of the mutant to hybridize with the mutation probe as a result of the presence of a rare or new mutation (20, 23). The MTBDR*plus* assay was not able to detect the INH resistance in the two isolates and Rifampicin resistance from three isolates which were detected by MGIT 960 system. This could be due to mutations which have not been included in the strips or it could also be due to unidentified mutation therefore the results from this study shows evidence that the GenoType ® MTBDR*plus* Version 2.0 assay can be considered as an alternative to the conventional phenotypic DST for the detection of RIF, INH, and/or multidrug resistance directly from sputum samples. Besides identification of drug resistance to RIF and INH, Geno Type ® MTBDR *plus* assay may provide information that is necessary for the understanding the evolution of drug resistance.

CONCLUSION

The study demonstrated that molecular line probe assay was consistent with phenotypic drug resistance testing for detection of gene mutations associated with *Mycobacterium tuberculosis.* The concordance with conventional phenotypic DST methods and rapid turnaround time make line probe assays useful tests for the diagnosis and management of tuberculosis drug resistance in HIV endemic regions like Kisumu County. Molecular line probe assay should be adopted as a routine diagnostic assay for monitoring and detection of *Mycobacterium tuberculosis* drug resistance in Kisumu County in the context of improving policies and systems in the County's path towards achieving and sustaining universal health coverage.To achieve Sustainable Development Goal target 3.3 which includes ending the TB epidemic by 2030, there remains a significant gap in the development of diagnostics suitable for use at the point of care as the technologies currently under development are primarily molecular based.

Acknowledgements:

The authors wish to thank the Kenya Medical Research Institute for providing the laboratory infrastructure that supported this study

REFERENCES

1. Gupta RK, Lucas SB, Fielding KL, Lawn SD. Prevalence of tuberculosis in post-mortem studies of HIV-infected adults and children in resource-limited settings: a systematic review and meta-analysis. PubMed. 2015;29:1987–2002.
2. Smith J, Serebrennikova Y, Huffman D, Leparc G, García-Rubio L. A new method for the detection of microorganisms in blood cultures: Part I. Theoretical analysis and simulation of blood culture processes. The Canadian Journal of Chemical Engineering. 2008;86(5):947–59.
3. WHO. Global tuberculosis report 2018. Geneva,Switzerland: 2018.
4. WHO. Global Tuberculosis Report. Geneva,Switzerland: World Health Organization, 2020.
5. Abebe G, Abdissa K, Abdissa A, Apers L, Agonafir M, Colebunders R. Relatively low primary drug resistant tuberculosis in south-western Ethiopia. BMC Res Notes. 2012;5:225.
6. Singh A, Rajendra P, Viswesvaran B, Gupta N. Drug-Resistant Tuberculosis and HIV Infection:Current Perspectives. HIV/AIDS - Research and Palliative Care 2020:12 9–31. 2020.
7. Abhijeet S, Rajendra P, Viswesvaran B, Nikhil G. Drug-Resistant Tuberculosis and HIV Infection: Current Perspectives. HIV/AIDS - Research and Palliative Care 2020;2020:12.
8. Kidenya R, Webster E, Sehan B, Rodrick K, Robert N, Peck S, et al. Epidemiology and genetic diversity of multidrug-resistant tuberculosis in East Africa. Tuberculosis (Edinb). 2014;94(1).
9. Suchindran S, Brouwer E, Van Rie A. Is HIV Infection a Risk Factor for Multi-Drug Resistant Tuberculosis? A Systematic Review. PLoS ONE. 2009;4(5): e5561.
10. Dheda K, Gumbo T, Maartens G, Dooley KE, McNerney R, Murray M, et al. The epidemiology, pathogenesis, transmission, diagnosis, and management of multidrug-resistant, extensively drug-resistant, and incurable tuberculosis. The lancet Respiratory medicine. 2017;5(4), 291-360.
11. WHO. Thirteenth General Programme of Work, 2019–2023. Geneva. Geneva: World Health Organization, 2018.
12. MOH. National Tuberculosis, Leprosy and Lung Disease Annual Report. Government of Kenya, 2020.
13. Ogari C, Antony K, Nonoh J, Amukoye E. Prevalence and detection of drug resistant mutations in Mycobacterium tuberculosis among drug naïve patients in Nairobi,Kenya. BMC Infectious Diseases. 2019;19:279.
14. Nyamogoba H, Mbuthia G. Gender-age distribution of tuberculosis Among suspected tuberculosis cases in Western Kenya. Journal of medicine science. 2018;10.5455(8735).
15. WHO. The use of molecular line probe assays for the detection of resistance to second-line anti-tuberculosis drugs: policy guidance. Geneva, Switzerland: World Health Organization, 2016.
16. Cheesbrough M. District laboratory practice in tropical countries, part II. 2nd ed ed. New York2006. p. 41–3 p.
17. Mukati S, Julka A, Varudkar H, Singapurwala M, Agrawat J, D. B. A study of clinical profile of cases of MDR-TB and evaluation of challenges faced in initiation of second line Anti tuberculosis treatment for MDR-TB cases admitted in drug resistance tuberculosis center. Indian J Tuberculosis. 2019;66(3):358–63, doi:http://dx.doi.org/10.1016/j.ijtb.2016.11.031.
18. WHO. Guidelines for Surveillance of Drug Resistance in Tuberculosis. Geneva, Switzerland: 2015.
19. Sharma B, Bhandari S, Maharjan B, Shrestha B, Banjara M. Rapid detection of rifampicin and isoniazid resistant *Mycobacterium tuberculosis* using genotype MTBDRplus assay in Nepal. Int Sch Res Notices. 2014;2014:648294.
20. Bai Y, Wang Y, Shao C, Hao Y, Jin Y, Espinal M. GenoType MTBDRplus assay for rapid detection of multidrug resistance in Mycobacterium tuberculosis: a meta-analysis. PLoS One 11:e0150321 doi: 101371/journalpone0150321. 2016.
21. Ahmed S, Shukla I, Fatima N, Sumit K. Profile of Drug Resistant Conferring Mutations among New and Previously Treated Pulmonary Tuberculosis Cases from Aligarh Region of Northern India. International Journal of Mycobacteriology. 2018;IP: 41.89.197.2.
22. WHO. The use of molecular line probe assays for the detection of resistance to isoniazid and rifampicin: policy update. World Health Organization, 2016.
23. Abanda N, Djieugoué J, Lim E, Pefura-Yone E, Mbacham W, Vernet G. Diagnostic accuracy and usefulness of the Genotype MTBDRplus assay in diagnosing multidrug-resistant tuberculosis in Cameroon: a cross-sectional study. BMC Infect Dis. 2017;2017;17(1):379(doi:http://dx.doi.org/10.1186/s12879-017-2489-3).

****