Acta Scientific Microbiology (ISSN: 2581-3226)

Volume 7 Issue 11 November 2024

Research Article

Genotype MTBDRplus Assay Detection of Rifampicin and Isoniazid Resistant *Mycobacterium tuberculosis* in Morocco

Aainouss Achraf[1,2]*, Momen Ghizlane[2], Chaoui Imane[3], Lamaammal Abdelmajid[2], Chetioui Fouad[2], Messaoudi Malika[2], Mouslim Jamal[1], Khyatti Meriem[2] and El Messaoudi My Driss[2]

[1]Department of Biology, Laboratory of Ecology and Environment, Microbiology Unit, Faculty of Science Ben M'Sik, Hassan II University, Casablanca, Morocco

[2]Laboratory of Mycobacteria and Tuberculosis, Institut Pasteur du Maroc, Casablanca, Morocco

[3]Unité de Recherches Médicales et Biologiques, Département des Sciences du Vivant, Centre National de l'Energie, des Sciences et Techniques Nucléaires, Rabat, Morocco

***Corresponding Author:** Aainouss Achraf, Department of Biology, Laboratory of Ecology and Environment, Microbiology Unit, Faculty of Science Ben M'Sik, Hassan II University, Casablanca, Morocco.

DOI: 10.31080/ASMI.2024.07.1448

Received: September 02, 2024

Published: October 26, 2024

Abstract

Tuberculosis remains a major public health problem in Morocco. The rise of different drug-resistance forms, particularly multi-drug resistant tuberculosis, became an increasingly dangerous obstacle to effective management of TB on a global scale. Multidrug-resistant TB (MDR-TB) is defined as resistance to at least isoniazid and Rifampicin, the two most potent drugs used as first-line treatment. The purpose of this study is to evaluate the effectiveness of using the GenoType MTBDRplus assay for Mycobacterium tuberculosis resistance detection to the two most important first-line antituberculous drugs.

This prospective study was conducted in the mycobacteria and tuberculosis laboratory, at Pasteur Institute of Morocco. A total of 177 sputum specimens were collected from patients with suspected Drug-resistant in Morocco. All samples were tested by conventional DST and Genotype MTBDRplus.

The phenotypic DST results indicated that out of the 177 isolates, 89 (50.3%) were resistant to both INH and RIF, with 8 (4.5%) and 9 (5.1%) isolates showing monoresistance to INH and RIF, respectively. The GenoType MTBDRplus assay identified 11 (6.2%) isolates as INH mono-resistant, 3 (1.7%) as RIF mono-resistant, and 87 (49.2%) as MDR. Of the 89 phenotypically identified MDR strains, 87 (97.7%) were also identified as MDR by the GenoType MTBDRplus assay. When compared to phenotypic DST, the sensitivity and specificity of the MTBDRplus assay were 90.8% and 98.7%, respectively, for detecting RIF-resistance, and 96.5% and 95%, respectively, for detecting INH-resistance.

In comparison with conventional DST, GenoType MTBDRplus assay was found to be an excellent tool for rapid diagnosis of MDR-TB cases in Morocco. The fast screening time for DR-TB samples is very helpful in stopping the spread of MDR-TB, particularly in our community.

Keywords: Tuberculosis; Drug-Resistance; GenoType MTBDRplus; Sensitivity

Abbreviations

TB: Tuberculosis; MDR-TB: Multi-drug resistant tuberculosis; INH: Isoniazid; RIF: Rifampicin; WHO: World Health Organization; LIPA: Line Probe assays; DST: drug susceptibility testing; LJ: Loewenstein-Jensen; WT: wild-type; MUT: Mutation

Introduction

Tuberculosis (TB) remains a serious infectious disease and a major public health problem worldwide [1]. In Morocco, the incidence of the disease is particularly high, with approximately 94 new cases per 100.000 inhabitants, corresponding to about 29327 cases in 2021 [2]. The emergence of drug-resistant strains, particularly multidrug-resistant TB (MDR-TB), which is defined as resistance to at least Isoniazid and Rifampicin, the two most potent drugs used in first-line treatment, poses a significant challenge to the national TB control program [3]. According to global surveys, the prevalence of MDR-TB strains was 1% among new cases and 8.7% among patients with a history of previous anti-TB treatment [2]. *Mycobacterium tuberculosis* drug resistance occurs due to spontaneous mutations in genes encoding drug targets, in regulatory regions of the target genes and in drug-activating genes [4]. The World Health Organization has recommended phenotypic drug susceptibility testing (DST) on solid media as the gold standard for detecting drug-resistant tuberculosis (DR-TB) [5]. However, these methods have a long turnaround time of 4 to 6 weeks, which may delay the detection of drug resistance and increase the risk of treatment failure and spread of drug-resistant strains [6].

In order to overcome the limitations of phenotypic methods, WHO has endorsed the use of molecular-based assays, such as Line Probe assays (LiPAs), for rapid detection of *M. tuberculosis complex* and its resistance to rifampicin and isoniazid [7]. The Genotype MTBDRplus (Hain Lifescience GmbH, Germany) uses multiplex polymerase chain reaction (PCR) in combination with reverse hybridization on nitrocellulose strips to detect mutations in loci of the 81-bp hotspot region of *rpoB* (which codes for the β-subunit of RNA polymerase), associated with rifampicin resistance. It also detects mutations at the codon 315 of the *katG* gene encoding for catalase-peroxidase to identify high-level isoniazid resistance, and mutations in the promoter region of *inhA* gene which encodes for NADH enoyl ACP reductase to identify low-level isoniazid resistance. Therefore, this study aimed to determine the patterns of mutations in *rpoB*, *katG*, and *inhA* in *Mycobacterium tuberculosis* Moroccan isolates, and evaluate the performance of the MTBDRplus assay in detecting drug resistance to rifampicin and isoniazid, compared to conventional drug susceptibility testing (DST).

Materials and Methods

Clinical specimens

The present study was conducted as a retrospective analysis at the Regional Reference Laboratory of Mycobacteria and Tuberculosis at the Pasteur Institute of Morocco (Casablanca, Morocco). From 2017 to 2021, 177 strains of *M. tuberculosis*, isolated from patients with pulmonary tuberculosis, were subjected to drug susceptibility testing (DST), and the results were compared to data obtained from the Genotype MTBDRplus assay. The sampling and testing of all isolates were performed as part of routine clinical practice for the patient's benefit, thus, approval from institutional ethics committee as well as informed consent were not required. The isolates were initially obtained from patients with a higher risk of drug-resistant TB. The *M. tuberculosis* H37Rv strain was used as a control for both techniques.

Samples processing and culture

The clinical samples were decontaminated using the Petroff method, which involved the addition of an equal volume of 4% NaOH to the sputum. The mixture was then homogenized and transferred to a Corning 50 mL centrifuge tube. Afterward, the samples were subjected to shaking in a Kahn agitator for 15 minutes. The resulting solution was then centrifuged at 3000 rotations per minute (rpm) for 20 minutes to achieve fluidification and decontamination. After completing this step, the supernatant was discarded in a bleach receptacle, while the sediment was mixed with 20 mL of sterile distilled water. Following this, the suspensions were centrifuged for 15 minutes at 3000 rotations per minute (rpm). The pellets were re-suspended in 1.5 mL of sterile distilled water. One portion of the suspension was subjected to routine culture using Loewenstein-Jensen medium, while the other portion served for GenoType MTBDRplus test (500 µL).

Mycobacterial culture of samples

Aliquots of decontaminated specimens were inoculated in duplicate into LJ medium. Media were then incubated at 37 °C for up to 6 weeks. All isolates were identified as *Mycobacterium tuberculosis* by their slow growth rate, colony morphology and colony pigmentation [8,9].

Conventional drug susceptibility testing

The drug susceptibility testing (DST) for *M. tuberculosis* isolates was conducted using the proportion method on LJ medium. The critical concentrations for Isoniazid and Rifampicin were 0.2 µg/mL and 40 µg/mL, respectively. A final reading of the DST was done after six weeks of incubation at 37 °C.

In this method, the growth of *Mycobacterium tuberculosis* on a medium containing a critical concentration of the anti-TB drug being tested is compared with the growth on a control medium without the drug. Strains are considered resistant if at least 1% of growth is observed at the critical concentration of the anti-TB drug in the culture medium [10].

GenoType MTBDRplus

The GenoType MTBDRplus line probe assay (LIPA) was carried out following the guidelines provided by the manufacturer (HainLifescience, Nehren, Germany) [4]. The assay consisted of three main steps, extracting DNA directly from the specimen sediment or indirectly from LJ culture using the GenoLyse kit; multiplex polymerase chain reaction (PCR) amplification, and reverse hybridization. Hybridized amplicons were detected using a colorimetric method involving streptavidin conjugated with alkaline phosphatase and a substrate buffer. The GenoType MTBDRplus examines each gene by checking for the existence of wild-type (WT) and mutant (MUT) probes. The wild-type probes target the crucial regions responsible for resistance in the respective gene, while the mutant probes are designed to detect commonly observed mutations associated with resistance [11-13].

To detect resistance to RIF, a total of eight wild type probes covering the *rpoB* gene region ranging from 504 to 533 were blotted along with four mutant probes named *rpoB MUT1 (D516V), rpoB MUT2A (H526Y), rpoB MUT2B (H526D),* and *rpoB MUT3 (S531L)*. For identifying INH resistance, one wild type probe covering codon 315 of the *katG* gene and two mutant probes named *katG MUT1 (AGC315ACC)* and *katG MUT2 (AGC315ACA)* were used. Furthermore, two wild-type probes that targeted the *inhA* gene were incorporated, along with four mutation probes, named *inhA MUT1 (C15T), inhA MUT2 (A16G), inhA MUT3A (T8C)*, and *inhA MUT3B (T8A).*

Susceptibility of the strain was inferred during interpretation if all wild-type probes were identified and no mutations were detected in the analyzed region. In contrast, if one or more wild-type bands were absent and/or one or more mutant bands were present, the sample was classified as resistant to the corresponding drug.

Results

Socio-demographic and clinical characteristics

A total of 177 suspected pulmonary TB patients were included in this study. The age of the patients ranged from 12 to 70 years old, the median age being 38. According to sex presentation, males were highly affected than females, with a sex ratio of 3.31 (76.8% vs. 23.2%). Among the 177 patients, 19.2% (34/177) were new TB cases, 22.6% (40/177) relapsed, 5.6% (10/177) failed to treatment, 13% (23/177) return after default whereas 27.1% (48/177) were still on TB treatment, 4% (7/177) were MDR-TB contact and 8.5% (15 /177) had unknown treatment history. The majority of patients were from the Casablanca-Settat region (173/177); (4/177) were from the Beni-MellalKhenifra region (Table 1).

Table 1: Socio-demographic and clinical characteristics of patients under the study.

Characteristics	n	%
Age, years		
2-15	2	1
15-25	25	14,3
25-45	89	50,5
45-60	52	29,5
>60	9	4,8
Gender		
Male	136	76,8
Female	41	23,2
Treatment history		
New case	34	19,2
Relapse	40	22,6
Failure	10	5,6
return after default	23	13
Still on treatment	48	27,1
MDR-TB contact	7	4
Unknown treatment history	15	8,5
Geographic region		
Casablanca - settat	173	97,8
Beni Mellal Khenifra	4	2,2

Conventional drug susceptibility testing results

Conventional DST was performed using the proportion method and the results showed that out of the 177 MTB specimens, 71 (40.1%) were susceptible to all three first-line antituberculosis drugs (INH, RIF, and Ethambutol). A total of 89 isolates (50.3%) were resistant to both RIF and INH whereas 5.1% and 4% were monoresistant to RIF and INH respectively.

GenoType MTBDRplus results

In this study, a total of 177 isolates were subjected to GenoType MTBDRplus testing, and the results are presented in Table 2. The findings revealed that 49.5% (87/177) of the isolates showed resistance to both RIF and INH, while 6.2% (11/177) were resistant to INH only, and 1.7% (3/177) were resistant to RIF only whereas 42.9% (76/177) of the isolates were sensitive to both RIF and INH.

Table 2: Conventional phenotypic DST for detection of RIF and INH resistance.

Susceptibility	Number of isolates (n = 177)	
	Genotype MTBDRplus (%)	LJ proportion DST (%)
RIF monoresistant	3 (1.7)	9 (5.1)
INH monoresistant	11 (6.2)	8 (4.5)
MDR-TB (resistant to RIF and INH)	87 (49,2)	89 (50.3)
Susceptibility to RIF and INH	76 (42.9)	71 (40.1)

Among nine phenotypically RIF mono-resistant isolates, only two harbored mutations in the 81-bp RRDR region of the *rpoB* gene. Among the eight specimens that were identified as being phenotypically resistant to INH, six had a mutation in only the *katG* gene. For the 89 MDR strains, 87 of them (97.7%) had known mutations in both rpoB and *katG* and/or *InhA* gene. It is important to mention that 76/177 isolates did not have any mutations targeted by the GenoType MTBDRplus test.

Mutations associated with rifampicin resistance:

Of the 90 RIF resistant TB strains detected by MTBDRplus assay, 58 (58/90; 64.4%) carried the Ser531Leu (*rpoB MUT3*) mutation, four strains (4/90; 4.4%) had a mutation at position H526D (*rpoB MUT2B*), and three strains (3/90; 3.3%) harbored GAC/GTC substitution in codon 516 *(rpoB MUT1)*. In this study, we identified two rifampicin-resistant strains with double mutations at two different codons of *rpoB* gene. One strain had *H526D* and *S531L* substitutions, while the other strain had *H526Y* and *S531L* mutations.

Out of the 90 strains, 25 (27.7%) were classified as having an unknown mutation due to the absence of the wild type (WT) pattern without any mutations detected by the probes. The majority of these strains (76%) were missing the WT8 probe, while 12% lacked the WT7 probe. Additionally, 2 strains each (8%) lacked the WT3, WT4, and WT5 probes, and only 1 strain (4%) lacked both the WT2 and WT3 probes.

Mutations associated with isoniazid resistance

Of the 98 INH-resistant strains detected by the Genotype MTBDRplus, 96 (98%) had a mutation in the *katG* gene, resulting in an amino acid substitution from serine to threonine at position 315, which indicates a high level of resistance. In our study, 19 out of 98 strains (19.4%) showed a mutation in the *inhA* promoter region, indicating low level resistance. Among the 96 strains with a *katG S315T* mutation, 17 (17.7%) had an additional mutation in the *inhA* promoter region. A total of 15 strains (88.2%) had *inhA MUT1* and two strains (11.8%) had *inhA MUT3A*. No single *katG MUT2, inhA MUT2*, or *inhA MUT3B* mutations were detected in our study population.

Mutations associated with multidrug resistance

Among 89 phenotypically MDR isolates, 87 were identified as Multi-drug resistant by the Genotype MTBDRplus assay. The predominant mutations observed in these MDR strains were located in codon 531 of the *rpoB* gene, associated with RIF resistance (S531L; 66.6%), and in codon 315 of the *katG* gene, involved in INH resistance (S315T1; 72.4%).

Performance of GenoType MTBDRplus

The study evaluated the performance of Genotype MTBDRplus compared to the phenotypic DST method, which is considered the "gold standard." The sensitivity and specificity of MTBDRplus for detecting RIF resistance were 90.8% [95% CI, 82.8-95.4%] and 98.7% [95% CI, 92.1-100%], respectively. For INH resistance, the sensitivity and specificity of MTBDRplus were 97% [95% CI, 90.5-99.1%] and 95% [95% CI, 87-98.4%], respectively. To detect Multidrug resistant TB, the MTBDRplus test had a sensitivity of 98% [95% CI, 91.3-99.6%] and a specificity of 97.7% [95% CI, 91.2-99.6%] (Table 3).

Table 3: Performance MTBDRplus in detecting INH resistance, RIF resistance and MDR-TB against phenotypic DST.

	Rifampicin	Isoniazid	Multi drug resistance
Sensitivity	90.8% (82.8-95.4)	96.5% (90.5-99.1)	97.7% (91.3-99.6)
Specificity	98.7% (92-99.9)	95% (87-98.3)	97.7% (91.3-99.6)
PPV	98.8% (93-99)	95% (89.2-98.6)	97.7% (91.3-99.6)
NPV	89.6% (80.8-94.8)	96% (88.5-99)	97.7% (91.3-99.6)

Discussion

Despite the efforts of the national tuberculosis control programs worldwide, TB continues to be a significant public health issue with a high incidence. The misuse of anti-TB drugs in chemotherapy, including inadequate treatment regimens, increases the risk of drug resistance emergence and spread [4]. Traditional drug susceptibility testing (DST) that uses solid/liquid media is a lengthy process since it requires bacterial culturing, which can take up to two months or even more. In 2008, the World Health Organization (WHO) approved the Genotype MTBDRplus assay for quick identification of patients at risk of developing MDR-TB. This molecular-based assay identifies the *Mycobacterium tuberculosis complex* as well as its resistance to rifampicin (RIF) and isoniazid (INH). The use of such rapid molecular assay is crucial for effective patient care and for decreasing the risk of relapse, failure treatment, and monitoring the spread of these resistant strains [14,15]. Hence, the objective of this research was to assess the diagnostic precision of Genotype MTBDRplus in quickly detecting resistance to RIF and INH, as compared to traditional techniques relying on sputum examination and culture either on solid or liquid media, and to monitor the resistance of *Mycobacterium tuberculosis* to the major first-line antituberculous drugs.

Genotype MTBDRplus for rifampicin resistance detection

The MTBDRplus assay showed a sensitivity of 90.8% and specificity of 98.7% for detecting resistance to RIF. Karimi., *et al*. also reported similar sensitivity and specificity values for RIF resistance detection in Tanger, Morocco [16]. However, our study found a lower sensitivity of the MTBDRplus for RIF resistance detection compared to Jian., *et al*. who reported a sensitivity of 98.7% [17]. Javed., *et al*. recently reported a sensitivity of 79.2% for the identification of Rifampicin resistance using the MTBDRplus assay [18]. In Ethiopia, another study reported a sensitivity and specificity of 80% and 99.6%, respectively, for detecting RIF resistance using the molecular test [19].

Molecular profile of rifampicin resistance

Approximately 72.2% of RIF-resistant isolates had known mutations. This finding is similar to the 73% value reported by *Singhal., et al.* [20]. The low detection rate of known mutations can be attributed to the fact that the Genotype MTBDRplus test is designed to detect only the four most frequent mutations in the RRDR region [17]. In our study, the most common mutation observed in the *rpoB* gene was the substitution of Serine with Leucine at codon 531, accounting for 64.4% (58/90) of cases. This result is in line with studies in Lithuania by *Bang., et al.* (61%) and *Barnard., et al.* (70.5%) [21,22]. *Sharma., et al.* reported a lower rate of the *rpoB MUT3* (*S531L*) mutation at 50% [23]. We also detected mutations in the *rpoB H526D* and *rpoB D516V* sites in 4.4% and 3.3% of RIF-resistant isolates, respectively, which is consistent with the findings in India reported by *Yadav., et al.* [24]. Our research found that out of the 90 RIF-resistant strains examined, 25 (equivalent to 27%) lacked one or more wild-type probes, despite having no known mutations. Other studies, such as singhal., *et al*. and Ennassiri*., et al.* have also reported similar results [20,25]. Also, the rate of mutations in the *rpoB* gene varies across different regions of the world, as documented in previous research [26].

Genotype MTBDRplus for isoniazid resistance detection

Our investigation found that the GenoType MTBDRplus assay showed a sensitivity of 96.5% and a specificity of 95% in detecting INH resistance. Likewise, a study by Aurin., *et al.* in Bangladesh reported that the MTBDRplus assay identified 99.6% of Isoniazid-resistant strains [12]. Furthermore, another research in Russia also reported comparable sensitivity to our study (97.1%) [26]. The latter study used conventional phenotypic susceptibility testing (DST) on liquid systems as the reference standard. Nevertheless, some other studies conducted in Ethiopia and Ghana reported lower INH sensitivity (91.7% and 83.3%), respectively [4,15]. The

relatively low sensitivity of the MTBDRplus test for detecting INH resistance can be attributed to the complex molecular mechanisms involved. This includes mutations across multiple genes or gene complexes, such as *katG, inhA, kasA*, and the intergenic region of the oxyR-ahpC complex [17].

Molecular profile of isoniazid resistance

Out of the 98 isolates that were resistant to Isoniazid detected by the GenoType MTBDRplus assay, 80.6% had mutations in only the *katG* gene, while 2% had mutations in the *inhA* promoter region, and 17.3% (17 isolates) showed mutations in both genes. Compared to similar studies, the frequency of each mutation detected in our research was lower than that reported in a previous study, which found 83% of *katG* mutations and 11% of *inhA* mutations [24]. Additionally, our study revealed a higher rate of samples with combined mutations in *katG* and *inhA* genes among INH-resistant isolates compared to a study by *Javed., et al.* conducted in Pakistan [18]. It is noteworthy that the reported frequency of *katG* and *inhA* gene mutations varies across different geographic regions [27].

Our study found that all 96 strains with a *katG* gene mutation had a *katGMUT1* (*S315T1*) mutation, indicating a 100% occurrence rate, which is in line with *Nikolayevskyy., et al.* findings in the Russian Federation [26]. However, *Maharjan., et al.* reported a lower frequency of *S315T1* mutation in the *katG* gene than our study, with a rate of 76.6% [28]. we found the presence of the C15T mutation in the *inhA* promoter region in 17% of the isolates, which matches the discovery of *Huyen., et al.* study in South Vietnam at 18%, and exceeds Sharma's findings of 3.1% [23,29]. In addition, our research identified two INH-resistant strains that exhibited mutations in *inhA MUT3A (T8C* mutation).

Detection of multidrug resistance using Genotype MTBDRplus assay

Our study found that the MTBDRplus assay had a sensitivity of 98% and specificity of 97.7% for detecting multi-drug resistant TB strains. In contrast, *Maharjan., et al.* reported a sensitivity rate of 88.7% and a specificity rate of 100% for the MTBDRplus [28]. Another study conducted in India also reported a sensitivity rate similar to the present investigation (97%) for the detection of multi-drug resistant TB using a molecular test, GenoType MTBDRplus [24].

Conclusion

The results of our study are representative of the general TB population, as the majority of the samples were collected from the Casablanca-Settat region, one of the most affected areas by tuberculosis at the national level. The study found that the GenoType MTBDRplus assay is an effective tool for the rapid diagnosis of MDR-TB cases in Morocco, compared to conventional DST. This molecular technique detects the most common mutations in specific regions of the *M. tuberculosis* genome, namely *rpoB, katG, and inhA genes*. The study highlighted the high frequency of mutations in the *katG* (S315T1) and *rpoB* (S531L) codons, associated with resistance to Isoniazid and Rifampicin, respectively. However, some discrepancies were noted between DST and molecular genotyping. Nevertheless, the short turnaround time for rapid screening of DR-TB isolates is an important tool in preventing the emergence and spread of DR-TB in the community.

The relatively low sensitivity to detect INH resistance for MTBDR*plus* is due to the complex molecular basis, which involves mutations in more than one gene or gene complex, such as the *katG*, *inhA*, and *kasA* genes and the intergenic region of the *oxyR-ahpC* complex.

Bibliography

1. El Hamdouni M., *et al*. "Treatment outcomes of drug resistant tuberculosis patients in Morocco: multi-centric prospective study". *BMC Infectious Disease* 19 (2019).

2. Ministère de la Santé du Maroc: Plan Stratégique national pour la prévention et le contrôle de la tuberculose au Maroc 2018-2021.

3. KHAZAEI S., *et al*. "Multidrug-Resistant TB as A Major Concern for Tuberculosis Control Programs". *Iranian Journal of Public Health* 45 (2016): 114-115.

4. Asante-Poku A., *et al*. "Evaluation of Genotype MTBDRplus for Rapid Detection of Drug Resistant Tuberculosis in Ghana". 12 (2016).

5. Bai Y., *et al*. "GenoType MTBDRplus Assay for Rapid Detection of Multidrug Resistance in Mycobacterium tuberculosis: A Meta-Analysis". *PLoS ONE* 11 (2016): e0150321.

6. Banu S., *et al*. "Discordance across Several Methods for Drug Susceptibility Testing of Drug-Resistant Mycobacterium tuberculosis Isolates in a Single Laboratory". *Journal of Clinical Microbiology* 52 (2014): 156-163 (2014).

7. Tilahun M., *et al*. "Molecular detection of multidrug resistance pattern and associated gene mutations in M. tuberculosis isolates from newly diagnosed pulmonary tuberculosis patients in Addis Ababa, Ethiopia". *PLoS One* 15 (2020).

8. Chikaonda T., *et al*. "Molecular characterisation of rifampicin-resistant Mycobacterium tuberculosis strains from Malawi". *African Journal of Laboratory Medicine* 6 (2017): 7.

9. Aung WW., *et al*. "Phenotypic and Genotypic Analysis of Anti-Tuberculosis Drug Resistance in Mycobacterium tuberculosis Isolates in Myanmar". *Annals of Laboratory Medicine* 35 (2015): 494-499.

10. Canetti G., *et al*. "Mycobacteria: laboratory methods for testing drug sensitivity and resistance". *Bulletin of the World Health Organization* 29 (1963): 565-578.

11. Crudu V., *et al*. "First Evaluation of an Improved Assay for Molecular Genetic Detection of Tuberculosis as Well as Rifampin and Isoniazid Resistances". *Journal of Clinical Microbiology* 50 (2012): 1264-1269.

12. Aurin TH., *et al*. "Molecular Approaches for Detection of the Multi-Drug Resistant Tuberculosis (MDR-TB) in Bangladesh". *PLoS ONE* 9 (2014): e99810.

13. Madhuri K., *et al*. "Utility of Line Probe Assay for the Early Detection of Multidrug-Resistant Pulmonary Tuberculosis". *Journal of Global Infectious Disease* 7 (2015): 60-65.

14. Kebede A., *et al*. "Performance of MTBDRplus assay in detecting multidrug resistant tuberculosis at hospital level". *BMC Research Notes* 10 (2017): 661.

15. Meaza A., *et al*. "Evaluation of genotype MTBDRplus VER 2.0 line probe assay for the detection of MDR-TB in smear positive and negative sputum samples". *BMC Infectious Disease* 17 (2017): 280.

16. Karimi H., *et al*. "Performance of GenoType® MTBDRplus assay in the diagnosis of drug-resistant tuberculosis in Tangier, Morocco". *Journal of Global Antimicrobial Resistance* 12 (2018): 63-67.

17. Jian J., *et al*. "Evaluation of the GenoType MTBDRplus and MTBDRsl for the detection of drug-resistant Mycobacterium tuberculosis on isolates from Beijing, China". *IDR* 11 (2018): 1627-1634.

18. Javed H., *et al*. "Evaluation of Genotype MTBDRplus and MTBDRsl Assays for Rapid Detection of Drug Resistance in Extensively Drug-Resistant Mycobacterium tuberculosis Isolates in Pakistan". F*rontiers in Microbiology* 9 (2018): 2265.

19. Bedewi Omer Z., *et al*. "Evaluation of the GenoType MTBDRplus assay for detection of rifampicin- and isoniazid-resistant Mycobacterium tuberculosis isolates in central Ethiopia". *International Journal of Mycobacteriology* 5 (2016): 475-481.

20. Singhal R., *et al*. "Frequency of multi-drug resistance and mutations in Mycobacterium tuberculosis isolates from Punjab state of India". *Journal of Epidemiology and Global Health* 7 (2017): 175-180.

21. Bang D., *et al*. "Performance of the GenoType MTBDRplus assay (v2.0) and a new extended GenoType MTBDRsl assay (v2.0) for the molecular detection of multi- and extensively drug-resistant Mycobacterium tuberculosis on isolates primarily from Lithuania". *Diagnostic Microbiology and Infectious Disease* 86 (2016): 377-381.

22. Barnard M., *et al*. "Rapid Molecular Screening for Multidrug-Resistant Tuberculosis in a High-Volume Public Health Laboratory in South Africa". *American Journal of Respiratory and Critical Care Medicine* 177 (2008): 787-792.

23. Sharma BK., *et al*. "Rapid Detection of Rifampicin and Isoniazid Resistant Mycobacterium tuberculosis Using Genotype MTBDR plus Assay in Nepal". *International Scholarly Research Notices* (2014): 1-6.

24. Yadav RN., *et al*. "Comparative Evaluation of GenoType MTBDRplus Line Probe Assay with Solid Culture Method in Early Diagnosis of Multidrug Resistant Tuberculosis (MDR-TB) at a Tertiary Care Centre in India". *PLOS ONE* 8 (2013): e72036.

25. Ennassiri W., *et al*. "Analysis of isoniazid and rifampicin resistance in Mycobacterium tuberculosis isolates in Morocco using GenoType® MTBDRplus assay". *Journal of Global Antimicrobial Resistance* 12 (2018): 197-201.

26. Nikolayevskyy V., *et al*. "Performance of the Genotype® MTBDRPlus resistance patternSamara, Russian Federation". *BMC Clinical Pathology* 9 (2009): 2.

27. Jaiswal I., *et al*. "Mutations in katG and inhA genes of isoniazid-resistant and -sensitive clinical isolates of Mycobacterium tuberculosis from cases of pulmonary tuberculosis and their association with minimum inhibitory concentration of isoniazid". *Clinical Epidemiology and Global Health* 5 (2017): 143-147.

28. Maharjan E., *et al*. "Use of Genotype MTBDRplus Assay for Diagnosis of Multidrug-Resistant Tuberculosis in Nepal". *International Scholarly Research Notices* (2017): 1-5.

29. Huyen MN., *et al*. "Validation of the GenoType® MTBDRplus assay for diagnosis of multidrug resistant tuberculosis in South Vietnam". *BMC Infectious Disease* 10 (2010): 149.