WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 10, 735-746. Research Article ISSN 2277– 7105

EVALUATION OF THE GENOTYPE MTBDRPLUS ASSAY FOR RIFAMPIN AND ISONIAZID SUSCEPTIBILITY TESTING OF *MYCOBACTERIUM TUBERCULOSIS* STRAINS IN SPUTUM SPECIME

Sita Ram Sapkota*, Bhawana Shrestha, Nikhil Ghimire and Pradeep Kumar Shah

Chhetrapati City: Kathmandu State: Bagmati Nepal.

Article Received on
15 August 2016,
Revised on 04 Sept. 2016,
Accepted on 24 Sept. 2016
DOI: 10.20959/wjpr201610-7084

***Corresponding Author**
Sita Ram Sapkota
Chhetrapati City: Kathmandu
State: Bagmati Nepal.

ABSTRACT

Rapid line probe assay (LPA) can be a practical and rapid alternative to the slow conventional phenotypic drug susceptibility testing (DST) for detection of drug resistant tuberculosis (TB). The purpose of this study is to determine the diagnostic accuracy of Genotype MTBDRplus, LPA for TB. A total of 85 culture samples were analyzed for evaluation MTBDRplus, assay A commercially available new Genotype MTBDRplus assay (Hain Lifescince, GmBH, Nehern, Germany) was evaluated for its ability to detect mutations in Mycobacterial isolates conferring resistance to rifampin (RMP) and isoniazid (INH). A total of 85 Samples were analysed in which 5 non tuberculoid mycobacteria strains, 55 multidrug resistant (MDR; i.e., at least resistant to RMP and INH), 24 fully susceptible strains were analyzed using Genotype MTBDRplus assay. MTBDRplus assay was designed to detect the mutations in the regulatory region of inhA. The MTBDRplus assay detected 55 of 80 RMP resistant strains (68.75%) with mutations on 81-bp hot spot region of rpoB gene and 48 of 50 of 2 INH resistant strains (96%) with mutation in codon 315 katG and regulatory region of inhA. In conclusion, the new Genotype MTBDRplus assay represents a rapid, reliable, upgraded tool for the detection of INH and RMP resistance strains that can readily be included in a routine laboratory work for the early diagnosis and control of MDR-TB.

KEYWORDS: MDR-TB, Genotype MTBDRplus assay, RMP, INH, rpoB, katG, inhA.

1. INTRODUCTION

Tuberculosis (TB) is chronic infectious disease caused by the bacterium *Mycobacterium tuberculosis* and characterized by the formation of granuloma in infected tissue as a result of

cell-mediated immunity. Based on the site of involvement of infection and host response, TB is of different types; pulmonary tuberculosis (PTB), extra pulmonary tuberculosis and disseminated tuberculosis (Cole *et al.,* 2004).Tuberculosis presents a significant health threat to the world's population, with 8 million new cases of disease and 2 million deaths per year (WHO, 2007). Drug –resistant TB is increasing in many parts of the world, and high rates of multidrug-resistant TB-causing (MDR-TB) isolates (resistant to at least isoniazid [INH] and rifampin [RMP] have been reported in several countries (Rieder *et al.,* 1996). 450,000 new multidrug-resistant TB (MDR –TB) cases are estimated to occur every year (WHO, 2007). One of the most alarming trends concerning tuberculosis is the emergence of drug-resistant *Mycobacterium tuberculosis* strains, which has become a global public health problem (Pablo's –Mendez *et al.,* 1998).

In Nepal about 40,000 people develop active TB with 20,000 infectious pulmonary diseases among which 5000 to 7000 people die of TB every year. The latest survey conducted in 2001/2002 showed MDR-TB of 1.3% of initial TB cases, 20.4% in acquired cases and 2.4% of TB patients where co-infected with HIV (NTC,2007).

Moreover, the problem of extensively drug resistant (XDR) strains has recently been introduced. These strains, in addition to being MDR, were initially defined as having resistance to at least two of the six main classes second-line drugs (Quinones, aminoglycosides) (CDC, 2006). Five cases of XDR-TB were recently reported in Nepal. XDR-TB now constitutes an emerging threat for the control of disease and future spread of drugs resistance, aspersing in HIV – infected patients, was reported (Gandhi *et al., 2006*). Due to this reason, rapid detection of drugs resistance to both first-and second line anti-tuberculosis drugs has become a key component of TB control programs.

Due to the high rates of MDR-TB isolates in Nepal, a research performed by Ghimire among the total studied cases, 12.9% isolates were resistant to one or more anti-tuberculosis drug (Rifampicin, Ethambutol, and Isoniazid &Streptomycin) with initial and acquired MDR 4.6% and 5% of the isolated respectively (Ghimire *et al., 2004)*, the present study aimed to determine the sensitivity and specificity fo MTBDRplus assay for the rapid detection of INH and RMP resistance-associated mutations in rpoB, katG and inhA from culture specimens in Nepal.

2. MATERIALS AND METHODS

2.1. Participants

This study was conducted in German Nepal Tuberculosis Project (GENETUP), national reference laboratory for The referred samples were the suspected multi-drug resistant TB patients visiting different DOTS plus centers of Nepal. A total of 85 sputum specimens were collected.

2.2. Test Methods

Specimens were obtained in sterile, leak proof, wide mouth, transparent, and stopper plastic containers. Fluorescence microscopy of the collected isolates was performed and all acid fast positive samples were cultured on Lowenstein-Jensen (LJ) medium. Culture positive samples were assessed for drug resistance using MTBDRplus assyT and molecular genotypic assay where the former is considered as gold standard for evaluation purpose.

Genotype MTBDRplus assay was also performed on mycobacterial cultures according to manufacturer's (Hain Lifescience) instructions. Briefly, genomic DNA was extracted from bacterial culture by suspending few colonies in 300 µL of molecular biology grade water and incubated for 20 minutes at 95°C in water bath and further 15 minutes in an ultrasonic bath with final spinning for 5 minutes at a speed of 12000 rpm. Polymerase chain reaction (PCR) was performed using primers and deoxyribonucleotide precursors provided by manufacturer and subsequent hybridization was done using the Twin-Cubator (Hain Lifescience) according to manufacturer's recommendations. Hybridized amplicons were colorimetrically detected using streptavidin-conjugated with alkaline phosphatase and substrate buffer. Finally, strip containing hybridized amplicons were air dried and fixed on evaluation paper for interpretation of drug resistance patterns of the isolates. During interpretation, an isolate was considered sensitive when all wild type probes produce band but no such bands in mutation probes. Missing of band development in any of the wild types probes or band development in any of the mutation probes suggests resistant type of isolates. Strips which tested positive for probes of amplification control, conjugate control, and locus control of targeted gene were only interpreted which otherwise are considered invalid.

3. RESULTS

3.1. Participants

In this study a total of 85 sputum samples referred from different DOTS plus centre of Nepal at National TB reference lab of GENEUP, Kalimati Kathmandu were drugs susceptibility

testing by Genotype MTBDRplus assa Out of total 85 sputum specimens referred to GENETUP by regional tuberculosis centres collected from MDR TB patients from different development regions, 80 samples were detected as MDR case MTBDRplus assay remaining 5 samples were detected as Non MTB.(Non mycobacterium Tuberculosis). Out of total 80 MDR- TB suspects 65(81.25%) were male and 15 (18.75%) were female. Out of 85 sputum samples only 80 were identified as MTB complex cases in which 58 were central , 6 were from eastern, 11 were from western, 6 were from mid-western, 3 were from far-western regions respectively. Similarly percentage wise distribution of MDR cases accounts as 72% from central, 11% were from western,7% were from eastern, 7% were from mid-western and 3% were from far-western development regions respectively (Table 1, Fig2).

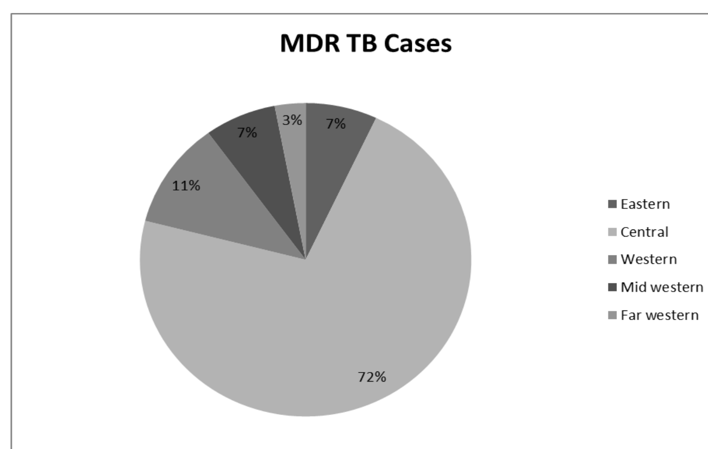

Figure 1: Flow diagram of positive TB cultures (a) GenoType MTBDRplus assy

Table 1: Gender of MDR Patients

Sex of MDR patients	No. of Patients	Percentage (%)
Male	65	81.25
Female	15	18.75
Total	80	100.00

3.2. Test Results

Table 2: Pattern of amino acid change and mutation on rpoB Gene

No(%) of Isolates	Amino acid change on rpoB gene	MTBDRplus assay RMP pattern (rpoB gene)	Result
40(50%)	rpoBS531L(Ser-Leu)	ΔWT8,MUT4	RMP^r
4(5%)	rpoBD516V(Asp-Val)	ΔWT3,4,MUT1	RMP^r
1(1.25%)	rpoBH526Y(His- Tyr)	ΔWT7,MUT2	RMP^r
1(1.25%)	rpoBH526D(His-Asp)	ΔWT7,MUT3	RMP^r
9(11.25%)	Not known	Δ(WT7,WT8,WT2,3,WT4)	RMP^r
2531.25%)	No change	WT	RMP^s

Table 3: Pattern of Mutation on rpoB Gene

Pattern of mutation	No. of Isolates	Amino acid changed
Δ WT8	2	Not known
WT	25	No change
Δ WT8, MUT4	40	rpoBS531L(Ser-Leu)
ΔWT7	1	Not known
ΔWT7, MUT2	1	rpoBH526Y(His-Tyr)
ΔWT 7, MUT3	1	rpoBH526D(His-Asp)
ΔWT 4	1	Not known
ΔWT4,5, MUT4	1	Not known
ΔWT3,4	1	Not known
Δ WT3,4, MUT1	4	rpoBD516V(Asp-Val)
ΔWT2,	1	Not known
ΔWT	2	Not known
Total	80	

Mutation in katG codon315 was detected in 48 of the 80 MDR isolates of which 48 isolates carried the ACC (S315T1 Ser- Thr) mutation (Table 4 and 5). 30 isolates had not shown any mutation pattern on the katG 315 codon.

Table4: Pattern of Amino Acid Change and Mutation on katG Gene

No(%) of Isolates	Amino acid change on katG gene	MTBDRplus assay INH pattern(katG gene)	Result
48(60%)	katGS315T1(Ser-Thr)	ΔWT,MUT1	INH^r
2(2.5%)	Not known	ΔWT	INH^r
30(367.5%)	No change	WT	INH^s

Table5

Table5: Pattern of Mutation on katG Gene with Amino Acid Change on katG Gene

No(%) of Isolates	Amino acid change on katG gene	MTBDRplus assay INH pattern(katG gene)	Result
48(60%)	katGS315T1(Ser-Thr)	ΔWT,MUT1	INH^r
2(2.5%)	Not known	ΔWT	INH^r
30(367.5%)	No change	WT	INH^s

Among the 6 (7.5%) of the MDR isolates showed a wild-type hybridization pattern in the katG 315 genetic code revealed mutation pattern in the ribosome binding site region of inhA. Among them 6 carried specific mutation on inhAC15T probe with omission of wild type 1 and next had deletion of wild type 2 without specific mutation band (Table 6 and 7).

Table6

Table6: Pattern of Amino Acid Changed and Mutation on inhA Gene

No(%) of Isolates	Nucleotide/amino acid change on inhA gene	MTBDRplus assay INH pattern (inhA gene)	Result
6(7.5%)	inhAC15T	ΔWT1,MUT1	INH^r
1(1.25%)	No change	ΔWT2	INH^r
73(91.25%)	No change	WT	INH^s

Table7: Pattern of Mutation on inhA Gene with Amino Acid Change on inhA Gene

Pattern of mutation on inhA gene	Nucleotide/ amino acid change on inhA gene				Total
	No change	inhAC15T	inhAT8C	Not known	
WT	73	0	0	0	73
ΔWT1,MUT1	0	6	0	0	6
ΔWT2	0	0	0	1	1
Total	73	6	0	1	80

4. DISCUSSION

In our study, discordance of sensitivity of Genotype MTBDRplus with phenotypic DST was reported. Sensitivity In this study, 80 *M. tuberculosis* complex, 55MDR isolates from different DOSTs plus centre of Nepal. Genotypic analysis of RMP and INH resistance was carried out with the MTBDRplus DNA strip assay. The test is based on a multiplex PCR in combination with reverse hybridization done on membrane strips coated with target-specific oligonucleotides. The reverse hybridization is followed by biotin-streptavidin- mediated detection of hybridized PCR fragments. The presence of wild-type or mutated DNA sequences in the 81-bp hot spot region of rpoB ,codon 315 of katG and regulatory region of inhA gene is then shown by clear-cut hybridization signals on the membrane strips, which can easily be analyzed The Genotype MTBDRplus assay allows the rapid and specific detection of the most-frequent mutations leading to IHN and RMP resistance. Both the PCR technology and the reverse hybridization technique used for the test have been proven to be robust and reproducible, and the results are easy to interpret without the extensive expert knowledge required for the interpretation of real-time PCR data or DNA sequencing data. The new MTBDRplus assay can be applied directly to smear-positive specimens, with a turnaround time of approximately 6 h, and save several weeks of time, which is required for primary isolation and conventional DST. Furthermore, it can be easily implemented in routine work flows, such as in differentiation of mycobacterium species and differentiation within the *M. tuberculosis* complex (Makinen *et al.*, 2002)).

Regarding RMP resistance, mutation in the rpoB gene was detected in 55of 80 MDR isolates (68.75%), whereas none of the 25 susceptible isolates carried a mutation in this region. Altogether, 13 different MTBDRplus hybridization patterns representing a minimum of 12 different mutations in the rpoB hot spot region were found. Codon 531 was most frequently affected in 31 of the 55 isolates (56.36%), with 12 strains showing the specific nucleotide exchange TCG to TTG, resulting in the amino acid exchange of serine to leucine. Five strains had a mutation located in the region from codons 531 to 535. However, this mutation was not further specified by the strip assay since the corresponding mutation was not targeted by an oligonucleotide on the strip. Other mutations were detected in rpoB codon 526 in 2 strains (3.63%), and rpoB codon 516 in 4 isolates (7.27%). In 6 of these strains, the particular mutations were clearly identified directly by hybridization to a specific oligonucleotide targeting the mutated sequence, and in 7 strains, they were identified indirectly by omission of specific wild-type signals. In two isolates hybridization patterns showing the missing wild-type pattern indicating the presence of mutated DNA sequences.

Overall, the Genotype MTBDRplus assay detected 55 of 80 (68.75.%) possible mutations conferring RMP resistance. 2 isolates (3.63%) phenotypically resistant to RMP were classified as susceptible by Genotype MTBDRplus assay due to the presence of a hybridization pattern typical for the wild type sequences in the rpoB hot spot region. These strains carried mutation in codon outside the 81 bp hot spot region of rpoB gene, which was not included in the DNA strip.

Khadka et al. (2011) attempt was to find out the base line information on the rpoB, KatG, and inhA mutation genes in M..tuberculosis strains isolated from re-trated cases in Nepal . These findings may be helpful for National Tuberculosis Control Program in developing policies for effective treatment and control of MDR-TB in Nepal. Furthermore, these studies in future. Through the rapid genotype test kits used in this study reportedly covers only common mutations regions, it is rapid, reliable and useful for quick detection of resistant mutation in rpoB gene, KatGgene and inhAgene and helpful in the early diagnosis of MDR-TB which in turn, help in the management of high burden MDR-TB cases .

RMP resistance was correctly identified in 39 of 41 isolates (95.1%) and 27 of 37 INH-resistant isolates (73%) with mutations in katG codon 315 with the Genotype MTBDR assay probes specific for these mutations, in the study carried out by Cavusoglu *et al,*. (2006) in Turkey.

Concerning INH resistance, in this study a mutation in katG codon315 was detected in 48 of the 50 MDR and 2 INH resistant isolates (96%) but in none of the 30 susceptible strains. In the majority of MDR isolates distinct nucleotide exchanges in katG codon 315 was verified by hybridization to the oligonucleotide probe targeting the particular mutations. 48 isolates carried the ACC (S315T1), and none carried the ACA (S315T2) mutation. In 2 further isolates (4%) mutation in codon 315 was indicated by the absence of the wild-type hybridization signal only.

In the present study, 7 (8.75%) of the MDR isolates showed a wild-type hybridization pattern in the katG 315 genetic code and revealed mutations pattern in the ribosome binding site region of inhA. Among them 6 (7.5%) carried specific mutation on inhAC15T probe with omission of wild type 1 and one (1.25%) carried mutation due to deletion of wild type 2 without specific mutation band.

The Genotype MTBDRplus assay has some limitations for the detection of antibiotic resistance and therefore cannot totally replace traditional culture-based methods for DST. This is basically due to the fact that none of the molecular tests established for targeting all possible genes or mechanisms (some are not identified yet) involved in resistance. Thus, a variable proportion of resistant isolates were not detected. These molecular tests should not be applied alone and therefore cannot totally replace culture based methods for several reasons: (i) apart from RMP and INH susceptibility testing, culture is needed for all other drug susceptibility tests (ii) RMP and INH susceptibility must be confirmed, since the possibility that a isolate is resistant cannot be excluded for a isolate with a wild-type pattern by the MTBDRplus assay (iii) in the case of a mixed infection with an MTBC isolate and a non tuberculoid mycobacterium, interpretation of the MTBDRplus assay results could be difficult (iv) furthermore, it should not be used as a nucleic acid amplification technique for the direct detection of MTBC strains in primary specimens. The low sensitivity for the detection of INH resistance was due to the fact that the test targets are only in the katG S315T mutation (Makinen *et al.,* 2006). By using the MTBDRplus assay, this problem is now at least partly solved by the addition of a second target for the detection of INH resistance, the regulatory region of the inhA gene.

Despite the clear advantages that molecular methods offer for drug susceptibility testing, they all suffer from the problem that the genetic basis of resistance is not fully understood for all *M. tuberculosis* isolates. This means that detection of mtation associated with resistant is

clinically relevant, but a lack of mutation in the target gene does not necessarily mean that the organism is susceptible to the drug.

The Genotype MTBDRplus assay appears to be a valuable tool that allows the detection of resistant *M. tuberculosis* isolates within one working day and can be easily included in routine workflow. It is a major improvement among assays for the routine detection of RMP and INH resistant MTBC isolates, since with this rapid and reliable tool, the therapeutic management of patients can be optimized. We are familiar by the fact that MDR cases are highly infectious, hence the prevention of resistant strains is one of the big challenge for present and future in Nepal for which rapid diagnosis technique like Genotype MTBDRplus assay can be a useful one.

5. CONCLUSION

In conclusion, the Genotype MTBDRplus assay is based on nucleic acid amplification method representing rapid and reliable technique for the detection of RMP and INH resistant MDR-TB isolates.

REFERENCES

1. G. B. Migliori, R. Loddenkemper, F. Blasi, and M. C. Raviglione, “125 years after Robert Koch's discovery of the tubercle bacillus: the new XDR-TB threat. Is “science” enough to tackle the epidemic?” European Respiratory Journal, 2007; 29(3): 423–427. View at Publisher · View at Google Scholar · View at Scopus
2. M. C. Raviglione and I. M. Smith, “XDR tuberculosis: implications for global public health,” New England Journal of Medicine, 2007; 356(7): 656–659. View at Publisher · View at Google Scholar · View at Scopus.
3. A Wright, M. Zignol, A. Van Deun et al., “Epidemiology of antituberculosis drug resistance 2002-07: an updated analysis of the Global Project on Anti-Tuberculosis Drug Resistance Surveillance,” The Lancet, 2009; 373(9678): 1861–1873. View at Publisher · View at Google Scholar · View at Scopus.
4. S. H. Gillespie, “Evolution of drug resistance in Mycobacterium tuberculosis: clinical and molecular perspective,” Antimicrobial Agents and Chemotherapy, 2002; 46(2): 267–274,. View at Publisher · View at Google Scholar · View at Scopus.
5. D. Hillemann, M. Weizenegger, T. Kubica, E. Richter, and S. Niemann, “Use of the genotype MTBDR assay for rapid detection of rifampin and isoniazid resistance in

Mycobacterium tuberculosis complex isolates," Journal of Clinical Microbiology, 2005; 43(8): 3699–3703,. View at Publisher · View at Google Scholar · View at Scopus

6. D. Hillemann, S. Rüsch-Gerdes, and E. Richter, "Evaluation of the GenoType MTBDRplus assay for rifampin and isoniazid susceptibility testing of Mycobacterium tuberculosis strains and clinical specimens," Journal of Clinical Microbiology, 2007;45(8): 2635–2640, View at Publisher · View at Google Scholar · View at Scopus
7. J. K. Varma, D. Wiriyakitjar, S. Nateniyom et al., "Evaluating the potential impact of the new Global Plan to Stop TB: Thailand, 2004-2005," Bulletin of the World Health Organization, 2007; 85(8): 586–592,. View at Publisher · View at Google Scholar · View at Scopus
8. T. C. Victor, R. Warren, J. L. Butt et al., "Genome and MIC stability in Mycobacterium tuberculosis and indications for continuation of use of isoniazid in multidrug-resistant tuberculosis," Journal of Medical Microbiology, 1997; 46(10): 847–857,. View at Publisher · View at Google Scholar · View at Scopus
9. World Health Organization, Molecular Line Probe Assays for Rapid Screening of Patients at Risk of Multidrug-Resistant Tuberculosis (MDR-TB): Policy Statement, World Health Organization, Geneva, Switzerland, 2008.
10. D. I. Ling, A. A. Zwerling, and M. Pai, "GenoType MTBDR assays for the diagnosis of multidrug-resistant tuberculosis: a meta-analysis," European Respiratory Journal, 2008; 32(5): 1165–1174, View at Publisher · View at Google Scholar · View at Scopus
11. F. Bwanga, S. Hoffner, M. Haile, and M. L. Joloba, "Direct susceptibility testing for multi drug resistant tuberculosis: a meta-analysis," BMC Infectious Diseases, vol. 9, article 67, 2009. View at Publisher · View at Google Scholar · View at Scopus
12. S. E. Dorman, V. N. Chihot, J. J. Lewis et al., "Genotype MTBDRplus for direct detection of mycobacterium tuberculosis and drug resistance in strains from gold miners in South Africa," Journal of Clinical Microbiology, 2012; 50(4): 1189–1194, View at Publisher · View at Google Scholar · View at Scopus
13. P. E. Akpaka, S. Baboolal, D. Clarke, L. Francis, and N. Rastogi, "Evaluation of methods for rapid detection of resistance to isoniazid and rifampin in Mycobacterium tuberculosis isolates collected in the Caribbean," Journal of Clinical Microbiology, 2008; 46(10): 3426–3428. View at Publisher · View at Google Scholar · View at Scopus
14. B. Dahal, N. Adhikari, Y. Shah, R. Simkhada, B. Maharjan, and B. Shrestha, "Evaluation of Genotype MTBDRplus Assay for identifying Multidrug Resistant Mycobacterium

tuberculosis isolates in Nepal," Janaki Medical College Journal of Medical Science, 2013; 1(1): 30–37, View at Google Scholar

15. X. Ma, H. Wang, Y. Deng et al., "rpoB gene mutations and molecular characterization of rifampin-resistant Mycobacterium tuberculosis isolates from Shandong Province, China," Journal of Clinical Microbiology, 2006; 44(9): 3409–3412, View at Publisher · View at Google Scholar · View at Scopus
16. H. Traore, K. Fissette, I. Bastian, M. Devleeschouwer, and F. Portaels, "Detection of rifampicin resistance in Mycobacterium tuberculosis isolates from diverse countries by a commercial line probe assay as an initial indicator of multidrug resistance," International Journal of Tuberculosis and Lung Disease, 2000; 4(5): 481–484, View at Google Scholar· View at Scopus

A. Poudel, C. Nakajima, Y. Fukushima et al., "Molecular characterization of multidrug-resistant Mycobacterium tuberculosis isolated in Nepal," Antimicrobial Agents and Chemotherapy, 2012; 56(6): 2831–2836. View at Publisher · View at Google Scholar · View at Scopus

17. M. N. T. Huyen, E. W. Tiemersma, N. T. N. Lan et al., "Validation of the GenoType MTBDRplus assay for diagnosis of multidrug resistant tuberculosis in South Vietnam," BMC Infectious Diseases, 2010; 10(149): View at Publisher · View at Google Scholar · View at Scopus
18. D. Rienthong, S. Rienthong, C. Boonin, S. Woraswad, and Y. Kasetjaroen, "Rapid detection for early appearance of Rifampin and Isoniazid resistance in Mycobacterium tuberculosis," Siriraj Medical Journal, 2009; 61(1): 49–55, View at Google Scholar
19. R. N. Yadav, B. K. Singh, S. K. Sharma, 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, vol. 8, no. 9, Article ID e 72036, 2013. View at Google Scholar
20. J. Yue, W. Shi, J. Xie, Y. Li, E. Zeng, and H. Wang, "Mutations in the rpoB gene of multidrug-resistant Mycobacterium tuberculosis isolates from China," Journal of Clinical Microbiology, 2003; 41(5): 2209–2212, View at Publisher · View at Google Scholar · View at Scopus
21. R. Raveendran, C. Wattal, J. K. Oberoi, N. Goel, S. Datta, and K. J. Prasad, "Utility of GenoType MTBDRplus assay in rapid diagnosis of multidrug resistant tuberculosis at a tertiary care centre in India," Indian Journal of Medical Microbiology, 2012; 30(1): 58–63, View at Publisher · View at Google Scholar · View at Scopus

22. F. A. Drobniewski and S. M. Wilson, “The rapid diagnosis of isoniazid and rifampicin resistance in Mycobacterium tuberculosis—a molecular story,” Journal of Medical Microbiology, 1998: 47(3): 189–196, View at Publisher · View at Google Scholar · View at Scopus
23. S. B. Marahatta, S. Gautam, S. Dhital et al., “KatG (SER 315 THR) gene mutation in isoniazid resistant Mycobacterium tuberculosis,” Kathmandu University Medical Journal, 2011; 9(33): 19–23, View at Google Scholar · View at Scopus