*Kat*G 315 Mutation as a Molecular Determinant for Isoniazid Resistance in *Mycobacterium tuberculosis*

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ABSTRACT

Emergence of multidrug resistant tuberculosis (MDR-TB) and extensively drug resistant tuberculosis (XDR-TB) is one of the reasons why tuberculosis (TB) continues to cause great mortality and morbidity in less-developed countries. The development of rapid diagnostic methods targeting genetic mutations associated with resistance to the anti-tuberculous drugs is essential to fight this deadly pathogen. Isoniazid (INH) has been included in the multidrug regimens for the treatment of drug-susceptible TB for the decades. In the worldwide setting, isoniazid resistance was highly prevalent and was observed in one of every seven TB cases. Since *kat*G315 mutation is highly prevalent, the common mutation in the enzyme essential for the activation of the INH concerned with the mechanism of drug resistance and associated with high level resistance to INH, *kat*G315 mutation was necessary to be identified by molecular method as a molecular determinant of INH resistant *Mycobacterium tuberculosis*. The prevalence of *kat*G315 mutation in various countries was discussed in this report and a new molecular method for the detection of the mutation was proposed.

Keywords: *Kat*G 315 mutation, molecular determinant, isoniazid resistance, *Mycobacterium tuberculosis*

INTRODUCTION

Tuberculosis (TB) continues to cause great mortality and morbidity in less-developed countries although an effective drug regimen has been available for decades. The reason for high mortality is prevalence of TB in HIV/AIDS pandemic population and emergence of multidrug resistant TB (MDR-TB) and extensively drug resistant TB (XDR-TB). These two factors made the control of the TB complicated worldwide.[1, 2] About 85% of new cases of TB were in Asia and Sub-Saharan Africa with 8.8 million new cases of TB were reported in 2010 and 1.4 million deaths occurred due to TB worldwide.[3]

Conventional methods for detection of drug resistance were usually undertaken in the reference laboratory in case of poor resource country with the delay of 4 – 8 weeks to get the results. Within that period, the patient was not properly treated with consequence of acquiring more serious drug resistance and having problem of unnecessary toxic effects of drugs, especially in HIV patients superimposed by TB. Rapid detection of drug resistance and starting of the correct treatment could overcome spread of multidrug resistant pathogens in the community and these measures are the top priorities in TB control. The development of rapid diagnostic methods targeting genetic mutations associated with resistance to the anti-tuberculous drugs in the tubercle bacilli is beneficial to fight this deadly pathogen.[4, 5] Studies of mutations and Single Nucleotide Polymorphisms (SNPs) within the genes associated with drug resistance were the research area which comes to the frontier in the control of TB.

History of Drug Resistance in *Mycobacterium tuberculosis*

Two anti-TB drugs, para-aminosalicylic acid and isoniazid (INH) were discovered in 1946 and 1952 respectively.[6, 7, 8] Although both of

the drugs were active against *Mycobacterium tuberculosis* (*M. tuberculosis*), drug resistance emerged within short period during the clinical usage of single drug.[9, 10, 11] Drug resistance has emerged to these two drugs in a short time because the drugs were used singly instead of combination with other anti-TB drugs. These two drugs, together with streptomycin (SM), which was discovered earlier, were combined in a first successful multidrug regimen for TB.[12] This three drug regimen was very effective that it was thought a foe of man has been already fought. The limitation of this regimen was long and expensive so that dropped out cases among patients made the therapy incomplete and problem of drug resistance emerged. In 1984, a new short-course treatment was started and this regimen had advantages of improved efficacy and better compliance of patients. The short course includes four drugs for 2 months followed by two drugs for 4 months. Four drugs were INH, rifampicin (RIF), pyrazinamide (PZA), and ethambutol and two drugs included INH and RIF. Strains resistant to INH and RIF started to emerge in 1985. To date, 20% of previously treated TB cases are caused by MDR-TB whereas nearly 4% of new cases were infected by these strains.[13] The *M. tuberculosis* strains which are resistant to INH, RIF, fluoroquinolones and one second-line drug that has to be given by injection are called XDR-TB. XDR-TB isolates are observed in at least 84 countries and prevalent up to 9% of MDR-TB. In addition, totally drug-resistant (TDR-TB) *M. tuberculosis* strains have already emerged in India, Iran, Africa and Europe and these strains are characterized by resistance formation to 10 TB drugs.[14] The reason for the starting and spreading of drug resistance is the delay in diagnosis because of unavailability of rapid diagnostic facilities.[15] Drug resistance mechanisms are essential for the diagnosis of these XDR-TB and TDR-TB. For MDR-TB, drug resistance mutations are well known.

INH Resistance in *M. tuberculosis*

INH is the prodrug and catalase peroxidase (*kat*G) activates INH which reacts with NAD+ resulting in INH-NAD. This compound inhibits InhA with the consequence of stoppage of mycolic acid biosynthesis which ends in mycobacterial cell death. SNPs in *kat*G gene result in inactive *kat*G with loss of activation of INH. S315T mutation of *kat*G was observed in 94 – 95% of INH-resistant clinical isolates.[15]

INH was synthesized in 1912 and its anti-tuberculous activity was known after 40 years. It has been included in the multidrug regimens for the treatment of drug-susceptible TB. In addition, INH monotherapy has been recommended for the management of latent TB. INH preventive therapy was well effective for HIV-infected individuals. In the worldwide setting, INH resistance was highly prevalent and was observed in one of seven TB cases.[16] In this review prevalence of *kat*G 315 mutations and other *kat*G mutations for INH resistance in different countries will be described.

*Kat*G Mutation in Multiple Drug-resistant Strains and Isoniazid Mono-resistant Strains

Fifty multiple drug-resistant, 50 INH mono-resistant and 50 susceptible strains of *M. tuberculosis* from the National Tuberculosis and Lung Diseases Research Institute in Warsaw, Poland were investigated for the prevalence of isoniazid resistance-associated mutations. Mutation distribution patterns between INH-monoresistant and MDR strains were compared in this study.

Of 109 INH resistant isolates, *kat*G 315 was observed in 46 isolates of the MDR strains, 31 of the INH-monoresistant strains whereas it was present in 2 pan-susceptible strains. G944C mutations at nucleotide level resulting in Ser315Thr (S315T) amino acid change was found in 33 MDR and 21 INH-monoresistant strains. G944C and C945T substitutions were coexistent in three of the isolates resulting in

same S315T mutation. G383A (Arg128Gln) and C701G (Ala234Gly), G1388T (Arg463Leu) mutations were observed in more than one isolate in MDR strains whereas A1197G (Glu399Glu) was present in three mono-resistant strains.[16]

*Kat*G Arg463Leu more Common than *kat*G Ser315Thr in Taiwan

In the study in Taiwan, the results were different with the observations in the other studies. Seventy *M. tuberculosis* isolates collected during the period from 1999 to 2011 were included in the study with the observation of 41 INH resistant isolates among 46 drug resistant isolates. Mutations were *kat*G Arg463Leu (R463L) (51%), S315T (29%), Ser315Asn (S315N) (9.8%), and other loci (22%). The canonical mutation S315T was relatively uncommon when compared with the other studies. This result makes the GenoType MTBDR plus molecular technique unreliable as this molecular diagnostic method used the *kat*G S315T as the probe (see Table 2).[17] However, the most prevalent mutation observed in the study, *kat*G R463L did not correlate with INH resistance as shown by the study in Netherlands where the mutation was nearly equalled in proportion among INH-susceptible isolates and INH resistant isolates.[18] In addition, the activity of the catalase-peroxidase in *M. tuberculosis* was not significantly changed by R463L which is induced in site-directed mutagenesis.[17]

*Kat*G315 Mutations in 49 Countries: A Literature Review

Although more than 95% of RIF resistance is associated with mutations in Rifampicin Resistance determining region (RRDR) which is 81bp region of the *rpo*B single gene, INH resistance is associated with mutations in multiple genes.[19, 20, 21, 22, 23, 24, 25] The existing molecular diagnostic methods for rapid detection of INH resistance have focused on the identification of the "canonical" mutations which are *kat*G codon 315 and *inh*A promoter region -15 nucleotide. However, the common one of the two canonical mutations was the point mutations in *kat*G codon 315. *Kat*G 315 mutations were prevalent up to 95% in the previous studies.[26, 27, 28] *Kat*G 315 mutation was averagely common up to 64.2 % of 8416 INH resistant isolates and 0.1% of 2462 INH sensitive isolates. S315T mutation was the most common mutation among *kat* gene of INH resistant isolates with second most common mutation has been S315N in the *kat* gene.[29] These two mutations can be used as molecular determinants of INH resistance in most of the studies although their frequency did not reach 100% in most of the studies.

*Inh*A promoter region -15 mutation was the most common mutations in the *inh*A gene and it was common up to 19% together with other mutations in the promoter region of the gene.[29]

Of 4505 isolates which had change of nucleotide with consequent change of amino acid information in *kat*G 315 mutation, *kat*G S315T (AGC-ACC) was present in 93.4% of isolates, whereas Ser315Asp (S315D) was 3.6% and *kat*G S315T (AGC-ACA) was 1.6%. Other mutations occurred among less than 1% of the isolates. As a common sense, those mutations with one nucleotide change were more common than those with two nucleotide change. However, S315T (AGC-ACA) was more common than Ser315Ile (S315I) (AGC- ATC).[29]

Different *kat*G Mutation at other Codons in Malaysia

The study in HUSM, Kelantan for mutations in *kat*G gene for INH resistant genes indicated that mutations in *kat*G 315 codon were not observed. Of 9 drug resistant isolates, only four isolates were observed to have INH resistant phenotypes. One isolate of these four isolates has Gln247His mutation at codon 247, another one has Val61Gly mutation at codon 61 and one also has Ala62Thr mutation at codon 62. The other INH resistant isolate has no mutation in the amplified region of *kat*G gene or no mutation in the *inh*A gene or its promoter region or in the other genes in which mutation leads to

INH resistance by *M. tuberculosis*.[30] However, *kat*G Leu238Arg, Ser238Ala mutations were collectively observed in 7 isolates with no phenotypic resistance to INH.[30]

Whole Genome Sequencing (WGS) Approach for Detection of Drug Susceptibility in Myanmar

Myanmar is highly prevalent for MDR-TB and was included in high-burden tuberculosis (TB) countries. It is of no doubt that earlier detection of MDR-TB is important for the control of tuberculosis.[31] Well-resourced, low-TB burden countries have facilities for WGS and it was considered for the diagnosis of drug-resistant TB. However, in resource-limited, high-TB burden country like Myanmar, routine implementation was not yet planned. As the improvement of TB control should be adopted earlier in the countries in which the facilities are needed most, evaluation of the usage of WGS in the diagnosis of MDR-TB and XDR-TB was conducted.[32]

Moreover, drug susceptibility testing (DST) with conventional method is time-consuming and taking weeks due to the prolonged culture necessary in *M. tuberculosis* with subsequent phenotypic testing. For these reasons, molecular methods such as GenoType MTBDRplus v.2.0 and GeneXpert MTB/RIF have been established in Myanmar. However, these methods can detect drug resistant mutations for the limited number of anti-tuberculous drugs. Whole-genome sequencing (WGS) is the possible way to supersede these methods.[33, 34, 35] Fourteen MDRTB isolates were sequenced by WGS and the results were consistent with phenotypic drug susceptibility testing (DST). Of 14 MDRTB isolates, all the isolates were resistant to INH with the mutations observed were *kat*G315 in 10 isolates and *inh*A promoter mutation in 2 isolates. The rest of the two isolates had G299C mutation in *kat*G gene in one isolate and frame shift mutation in *kat*G gene in the other isolate. *Kat*G315 mutations in 10 isolates were the same with change from Serine to Threonine whereas *inh*A mutations were at −15 nucleotide in the promoter region (Table 1).[32]

INH Resistant Mutations in Brazil

MDR-TB isolates were randomly chosen and collected from Central Public Health Laboratory, State of Bahia, Brazil.[36] These strains were isolated from sputum samples collected from local patients. Molecular determinants for MDR-TB isolates commonly used in the previous studies and observed to be prevalent were S315T in *katG*, −15C/T in the promoter region of *inh*A, and H526D and S531L mutations in *rpoB* genes. Regarding *kat*G S315Tpolymorphism, it was observed to be 100% of INH resistant strains in some countries (Table 2)[37, 38, 39] and variable number of percentage in other countries whereas the study in Brazil indicated 41.9%. The −15C/T (*inhA*) polymorphism, the other mutation for INH resistance was observed to have frequency of 25.6% in the study. From the previous studies, there was an information that type of mutations and frequency of these mutations associated with drug resistance in *M. tuberculosis* varied according to the geographical regions.[40, 41]

***Kat*G 315 Mutations in African Countries**

A total of 63 drug resistant *M. tuberculosis* clinical isolates were screened for genetic mutations associated with INH, RIF, SM and Ethambutol resistance among the positive pulmonary tuberculosis patients enrolled from April 2010 and March 2011. Thirty two of 44 isoniazid resistant isolates were observed to have *kat*G315 and/or the −15 *inh*A promoter mutations. All the *kat*G315 mutations, three (−15C/T) *inh*A promoter mutation and 6 wild types exhibit high level drug resistance. The details were shown in Table 1 and described under the heading of Minimum Inhibitory concentration for INH resistant isolates.[42]

Between 2008 and 2011, two drug resistance surveys were conducted in Uganda by using WGS method. Of these two surveys, 90 *M. tuberculosis* isolates which are phenotypically resistant to RIF and/or INH were selected and sequenced for whole genome. Mutations observed were *kat*G S315T in 44 cases, S315N in 2 isolates, S315R in 2 isolates, S315T with

*inh*A promoter mutation −15 C/T in 1 isolate, S315T with *ahp*C 48 G/A mutation in 1 isolate. High-level INH resistance was observed in *kat*G codon 315 mutations whereas low-level resistance was associated with *inh*A promoter mutations. However, very high MIC to INH was found in isolates carrying both *kat*G315 and *inh*A promoter mutation. Furthermore, *kat*G mutations were associated with high incidence of tuberculosis with higher transmission rates and unfavourable outcome worldwide.[43]

Methodology Applied in Studying INH Resistant Mutations

In most of the studies, resistance to INH was detected on Lowenstein-Jensen medium by using agar proportion method with INH concentration of 0.2 mg/L. The MIC of INH was measured and determined by 2-fold incremental concentrations of INH starting from 0.05 mg/L, ending at 60 mg/L.[16]

Methodology for studying mutations includes genomic DNA isolation, PCR of DNA fragments flanking *kat*G 315 mutation and *inh*A mutations and sequencing of the PCR product using same PCR primers using ABI Big dye terminator sequencing kit.[16, 17, 30, 42] In the study in Taiwan, four overlapping pairs of forward and reverse primer pairs were applied for PCR reactions and 1710 bp length DNA sequence was studied to get the mutations of the *kat*G gene. Similarly, two overlapping sets of primers were used for 810 bp PCR product in the PCR for *inh*A mutations both in promoter region and open reading frame. Comparison was undertaken with the *Mycobacterium tuberculosis* reference strain H37RV to find out SNPs[17]. For the *kat*G 315 mutation, 210 bp was amplified and sequenced whereas in case of *inh*A promoter mutations, 248 bp PCR product was amplified and sequenced in the study of INH resistant mutations in Cameroon.[42]

WGS approach was used to study the resistant mutations to all anti-tuberculous drugs in Myanmar and Uganda after DNA extraction and purification as described in Aung et al.[32] and Ssengooba et al.[43].

Reasons of *kat*G Mutation is Common in some Countries and Rare in Other Countries

A conclusion was drawn in the study on isoniazid resistant *M. tuberculosis* in Brazil that type and frequency of the SNPs in *kat*G gene varied according to the geographical regions without the reasons to explain it. However, the following explanation can be undertaken. There were many genotypic lineages in *M. tuberculosis* infecting human worldwide such as Beijing, T- families, LAM, Haarlem, etc.[44]. In several countries, studies indicated *M. tuberculosis* isolates carrying the Beijing genotype had the *katG315* mutation associated with high-level resistance to INH when compared with other mutations. The Beijing genotype appears to develop this *kat*G mutation in comparison with other genotypes.[45] The genotypic lineage varies with the various regions of the world. Therefore taken together, the mutations associated with INH resistance varies with the spreading of *M. tuberculosis* genotypes in different countries. Another example is *kat*G R463L mutation is common in Netherlands and Taiwan although it is not associated with INH resistance as shown by experimental finding.[17, 18]

Table 1 INH resistant mutations in different countries with frequency of *kat*G 315 mutations and *inh*A mutations

Countries of study and no. of INH resistant isolates studied	***kat*G mutations and frequency of *kat*G S315T mutations**	**Mutations in *inh*A promoter region and *inh*A gene**
Poland (109 INH resistant isolates)[16]	*kat*G Ser315Thr, Arg128Gln, Ala234Gly, Arg463Leu, Glu399Glu 57 of 109 isolates	Mutations in the *inh*A promoter region were detected in eight MDR strains (−15C/T in seven strains and −8T/C in one strain). Mutations in the *inh*A gene were of four types.
Taiwan (41 INH resistant isolates)[17]	*kat*G Arg463Leu, Ser315Thr, Ser315Asn, and other loci 12 of 41 isolates	Only one isolate with *inh*A promoter mutation (−15C/T) was observed.
*Myanmar (14 INH resistant isolates)[32]	*kat*G Ser315Thr, Gly299Cys, frameshift. 10 of 14 isolates	2 isolates with *inh*A promoter mutation (−15C/T).
Malaysia (4 INH resistant isolates)[30]	*kat*G Gln247His, Val61Gly, Ala62Thr,	-
Cameroon (44 INH resistant isolates)[42]	*kat*G315 32 of 44 isolates	13 isolates with *inh*A promoter mutation (−15C/T).
*Uganda (50 INH resistant isolates)[43]	*kat*G315 Ser315Thr, Ser315Asn, Ser315Arg 50 of 50 isolates	9 isolates with *inh*A promoter mutation (−15 C/T) 1 isolate with *inh*A promoter mutation (−8T/C)

*Method of study is WGS.

Minimum Inhibitory Concentration for INH Resistant Isolates Carrying *kat*G Mutation

MIC was expressed in mg/L in some literatures and μg/ml in other literatures. In this report, mg/L will be used for simplicity purpose. In the study in Cameroon, the researchers divided the MIC into high level, 1 mg/L and low level, 0.2 mg/L. Twenty-four of 44 INH resistant isolates had 1 mg/L MIC showing high level drug resistance. Of these, 17 *kat*G S315T mutant isolates had high level drug resistance whereas 2 isolates showed low level resistance. Three isolates carrying (−15C/T) *inh*A promoter point

mutation displayed high level resistance and ten of these isolates showed low level resistance. Five (–47G/C) *inh*A promoter mutant isolates showed high level resistance with no low level resistance.[42] In the literature review in which INH resistance isolates in 49 countries were studied, it was observed that *kat*G codon 315 was associated with high level resistance to INH with 4 mg/L or more MIC value.[29] The finding in Warsaw, Poland indicated that *M. tuberculosis* isolates with *kat*G 315 mutants had the MIC values between 1 to 10 mg/L (Table 2) with the average MIC of 2.5 mg/L in the MDR and isoniazid mono-resistant phenotypic strains.[16]

Advantages and Limitations of Currently using Molecular Methods

Although there was variation of the sensitivity and specificity of the results in different regions of the world where these were applied, GeneXpert and GenoType MTBDRplus were relatively simple and extremely rapid in detection of drug-resistant TB.[4, 46] However, the main disadvantage of these molecular methods is resistance detection can be available for fewer drugs and limited number of mutation. GeneXpert cannot detect INH-monoresistant cases (Table 2) that might become MDR-TB in the future[47]. In most TB endemic countries, Mono-INHR tends to rise as a result of inability to capture these isolates by molecular methods.[48] Moreover, as observed in the previous studies S315T, a canonical mutation was lower in prevalence in INH mono-resistant isolates whereas it was highly prevalent in MDR-TB isolates.[49] Research on development of rapid diagnostics which can encompass novel mutations conferring drug resistant TB are essential.[49]

Table 2 Significance of *kat*G 315 mutations in INH resistant *M. tuberculosis*

In some countries, *kat*G 315 mutations were prevalent up to 100%.[37, 38, 39]
M. tuberculosis isolates with *kat*G 315 mutants were commonly associated with the MIC values between 1 to 10 mg/L showing high level INH resistance.[16]
*Kat*G 315 mutations were more common in MDR-TB isolates than INH monoresistant isolates.[16]
Of two canonical mutations, *kat*G 315 was the more common one than (−15C/T) *inh*A promoter mutation. Other mutations associated with INH resistance were not included in canonical mutations.[29]
Ser315Thr (AGC-ACC) was present in more than 90% in INH resistant isolates with *kat*G 315 mutations.[29]
Ser315Asp was the second most common mutation among *kat*G 315 mutations.[29]
*Kat*G 315 mutation was included in the GenoType MTBDRplus molecular diagnostic method.[17] The rapid molecular diagnostic methods come to the front line in the diagnosis of drug resistant TB because conventional methods for drug sensitivity test usually take 8 weeks in most regions and treatment of the patients will be delayed.[32]
*Kat*G 315 mutation was not detected in the GeneXpert MTB/RIF molecular diagnostic method.[49]

Discussion on Molecular Determinants in MDR-TB and XDR-TB

Although WGS is a perfect platform to detect MDR-TB as well as XDR-TB, implementation was still earlier for the routine purpose in the low resource-high TB burden countries. Findings of other molecular diagnostic methods are the main areas of research. Researchers in the drug resistant TB now focus on identification of mutations associated with drug resistance in the genome of *M. tuberculosis* and innovation of molecular diagnostics to detect these mutants. Accuracy up to 100% was not obtained in these methods because the association between the molecular determinants and the phenotypes of the isolates with these mutations is not strong enough.[50]

WGS approaches in Uganda and Myanmar indicated that *rpo*B S531L and *kat*G S315T were the highly prevalent molecular markers for MDR-TB isolates. In Myanmar, *rpo*B S531L was observed in 11 of 14 isolates with H526Y as the second common mutation. Regarding INH resistant mutation, *kat*G S315T was also found in 11 isolates whereas *inh*A C-15T promoter mutation was present in two isolates as the second common mutation. In Uganda, S531L and H526D were the most two common mutations for RIF resistance whereas *kat*G S315T and *inh*A C-15T promoter mutation were the most two common ones for INH resistance. Although these results were observed in these two studies, it was clear that *rpo*B S531L and *kat*G S315T are the pre-dominant molecular determinants for RIF and INH respectively. The outstanding characteristic of *kat*G S315T SNP in INH resistance is relatively more common than *rpo*B S531L in RIF resistance. This fact indicated that *kat*G S315T was a stronger marker for INH resistance than *rpo*B S531L in RIF resistance.[32, 43] The observations in other studies of various countries were consistent with these two studies. In conclusion, drug resistant molecular markers for MDR-TB were well established.

Besides mutations associated with RIF and INH, it is necessary to study drug resistant mutations commonly associated with resistance to fluoroquinolones and other second line drugs (SLD) to identify XDR-TB. Drug resistant mutations for SLD were less studied and less understood. Although detection of *gyr*A and *gyr*B mutations additively gave rise to sensitivity of 93% for the fluoroquinolone resistance, *gyr*B mutations were widespread and molecular determinants were inconsistently associated with drug resistant phenotypes. Taken together, these mutations were not reliable to prepare the rapid molecular diagnostics. In the study in France, *gyr*A mutants Ala90Val, Asp94Gly, Asp94Ala were common molecular determinants for fluoroquinolone resistance whereas 13 SNPs in *gyr*B were observed. In addition, these SNPs were observed to be present in fluoroquinolone sensitive *M. tuberculosis* isolates[51]. SNP *rrs* A1401G was highly prevalent in isolates resistant to these SLD which have to be given by injection like capreomycin (CAP), amikacin (AMK) and kanamycin (KAN). However, the *rrs* A1401G mutation was present only in 70 – 80% of *M. tuberculosis* strains resistant to CAP and AMK whereas this mutation was observed in 60% of strains resistant to KAN. MTBDRsl line probe assay (LPA) is based on the principle of hybridization and mutations in clinical strains were detected by the probes that are complementary to the mutated DNA. MTBDRsl LPA is the only available rapid molecular diagnostic method widely used for identification of XDR-TB. However, the method has variable sensitivity with the range from 40 – 100%.[50]

CONCLUSIONS

*Kat*G gene encodes catalase peroxidase which activates anti-TB drug INH and is an important enzyme for the survival of the bacteria in the macrophages and it is regarded as virulence factor of *M. tuberculosis*.[52]

Although *kat*G315 mutations has shown to be highly common up to 95% in the previous studies, other *kat*G mutations are found in the literatures and observed to be associated with INH resistance. In addition, *kat*G315 mutation was uncommon as low as 25% in INH monoresistant cases. It will be necessary for the researchers to develop the molecular methods which can include probes possible to detect all novel mutations.

Dissemination of MDR-TB and XDR-TB will be dangerous and cause high mortality in the TB endemic countries. In addition, second line TB drugs have higher toxicity and are expensive so that there may be many dropped out during the regimen which leads to increasing mortality. Therefore finding of hotspot areas and screening of hotspots within the localities in the poor resource countries with earlier diagnosis and rapid implementation of appropriate anti-tuberculous drug regime will be the essential

measure in the control of TB. Hotspot areas are defined as TB endemic localities with the prevalence rate of more than 0.5 – 1%.

In spite of the fact that *kat*G315 mutation is not the only mutation for INH resistance, it is highly prevalent and it is the common mutation in the main enzyme essential for the activation of the INH regarding the mechanism of drug resistance. Moreover, *kat*G S315T mutation has been shown to be associated with high level resistance to INH with average MIC of 1 ug/ml in the previous studies. As a consequence, rapid method of identification of this point mutation with mismatch amplification mutation assay (MAMA) is recommended in this report.

SNPs that are not detectable by other polymerase chain reaction or PCR-RFLP can be detected by MAMA PCR. One nucleotide change in quinolone resistance determining region of *gyr*A gene responsible for fluoroquinolone resistant bacteria such as *Klebsiella pneumoniae, Campylobacter jejuni* and *Neisseria gonorrhoeae* can be identified by PCR assays using MAMA method.[53] Discrimination of the *ctxB* alleles in classical, El Tor, and Haitian type *Vibrio cholerae* can be undertaken by Double-mismatch-amplification mutation assay (DMAMA) PCR.[54] The canonical mutations for INH resistance, *kat*G S315T mutation and (−15C/T) *inh*A promoter mutation, the two SNPs can be proposed to be detected by DMAMA.

In Malaysia, the study in Kelantan has shown totally different mutations in four cases with INH resistance. The common mutations were not observed in the study because the total number of samples studied was only nine. It will be interesting if a large survey of drug resistant *M. tuberculosis* isolates is undertaken in the near future within Malaysia.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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