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The Prediction Factors of Pre-XDR and XDR-TB among MDR-TB Patients in Northern Thailand

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Abstract

Background: Molecular diagnosis based on the detection of mutations conferring genetic drug resistance is useful for early diagnosis and treatment of Pre-XDR and XDR-TB patients. However, the study of mutation as a marker to predict Pre-XDR and XDR-TB is rare. **Methods:** Thirty-four *Mycobacterium tuberculosis* (MTB) isolates from MDR, Pre-XDR and XDR-TB patients in the upper north of Thailand, who had been identified for drug susceptibility using the indirect agar proportion method from 2005-2012, were examined for genetic site mutations of *katG*, *inhA*, and *ahpC* for isoniazid (INH) drug resistance, *rpoB* for rifampicin (RIF) drug resistance, *gyrA* for ofloxacin (OFX), and *rrs* for kanamycin (KAN). Associations between resistant genes and Pre-XDR and XDR-TB in the MDR patients were performed using exact probability tests. Univariable logistic regression was used to quantify the strength of association between the gene mutation with *Mycobacterium tuberculosis* and the prevalence of Pre-XDR and XDR-TB in the MDR patients. **Results:** The mutations in the region of the *rpoB* gene at codon 445 (C445T) in the Pre-XDR or XDR-TB patients were significantly 20.6 times more prevalent among the MDR-TB patients. The *inhA* gene mutation at codon 114 (T114G) was also significantly 8.1 times more prevalent. **Conclusion:** The findings can be used to predict the odds of Pre-XDR and XDR-TB in MDR-TB patients, as a guide for prevention and treatments.

Keywords

Prediction, Tuberculosis, Drug Resistance, MDR-TB, XDR-TB

1. Introduction

Extensively drug-resistant (XDR) tuberculosis (TB) has emerged as a major threat to global TB control. *Mycobacterium tuberculosis* XDR strains are resistant to rifampin, isoniazid, fluoroquinolone, and any of the second-line injectable agents, including amikacin (AMK), kanamycin (KAN), and capreomycin (CAP) [1]. XDR-TB is usually developed from multidrug-resistant (MDR) TB, which is resistant to rifampin and isoniazid. MDR-TB typically requires two years of treatment with second-line drugs, which is more expensive and more toxic than first-line drugs [2] [3]. The low rate of diagnosis and diagnostic delay, the limited access to second-line drugs, and the poor adherence of MDR-TB patients have mainly led to the emergence of XDR-TB [4]. Most of the XDR-TB and Pre-XDR-TB patients in China were new cases, indicating the transmission of resistant strains [5] [6]. In 2016, Thailand had 80 MDR-TB patients. Of these cases, 20 were on treatment for XDR-TB and 60 were on MDR-TB and Pre-XDR-TB medication [7]. All of them are difficult to treatment.

The cure rate for MDR-TB patients is 50% - 60%, compared with 95% - 97% of the patients with drug-susceptible TB [8]. As a result, MDR-TB and XDR-TB have emerged as significant threats to global TB control [9]. The emergence of XDR-TB strains is a reflection of poor tuberculosis management and control, and this situation should be considered as an urgent global health problem, especially in developing countries and those lacking resources [10].

Our study aimed to ascertain the risk factors of gene mutation that are associated with the development of Pre-XDR and XDR-TB. The rapid diagnosis of these resistant cases is urgently needed and is useful for treatment. In the future, molecular diagnosis will involve MDR-TB and XDR-TB detection, which is also useful for predicting Pre-XDR and XDR-TB and for monitoring treatment.

2. Material and Methods

2.1. Study Design

This study was a retrospective study of MDR-TB and XDR-TB *M. tuberculosis* isolates involving TB patients during 2005-2012 at the Office of Disease Prevention and Control Region 10 (DPC 10) in the north of Thailand as shown in **Figure 1**. The DPC 10 laboratory is a Regional TB Laboratory covering TB patient treatment from eight provinces in the upper north of Thailand, which can provide *M. tuberculosis* (MTB) cultures, identification, and Drug Susceptibility Tests (DST) for first- and second-line drugs. The MTB isolates were subcultured, then tested for phenotypes for first- and second-line drug resistance to isoniazid (INH), rifampicin (RIF), ofloxacin (OFX), and kanamycin (KAN) at DPC 10. Further, genetic site mutation for drug resistance in the corresponding resistant gene (*katG*, *inhA*, *ahpC*, *rpoB*, *gyrA* and *rrs*) was performed at Macrogen in Korea. Medical records were retrospectively reviewed for demographic data, diagnosis, and laboratory identification and DST results.

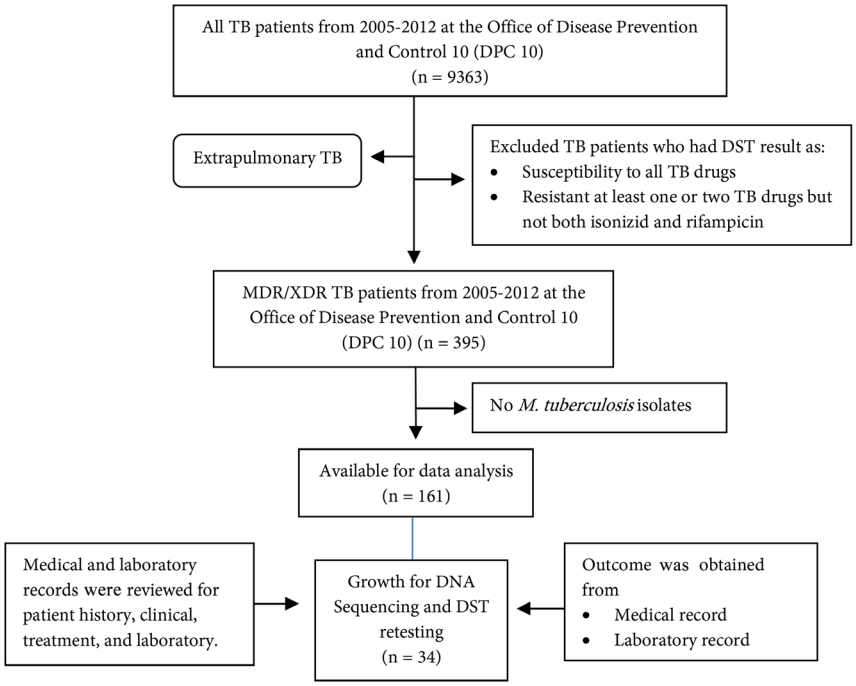

Figure 1. Study flow.

2.2. Mycobacterial Isolates

161 MTB multidrug and extensive drug resistant strain isolates from TB patients during 2005-2012 were subcultured from collections at DPC 10 received from 8 hospitals in the upper north of Thailand. Only 34 isolates were able to grow in 5 ml of 7H9 broth supplemented with PANTA and 3% Ogawa. Samples were collected from individual TB patients who presented with the initial treatment status.

2.3. Drug Susceptibility Testing

Phenotypes testing on first and second line anti-tuberculous drugs (INH, RIF, OFX, and KAN) were performed on 34 isolates of *M. tuberculosis*, using the proportion method on LJ medium [11]. DST was completed according to the WHO guideline for DST testing for first- and second-line anti-tuberculosis drugs for DOTS-plus [12]. DST was determined using the indirect agar proportion method which, was performed on an LJ medium supplemented individually with anti-TB drugs, which included INH (0.2 μg/ml), RIF (40.0 μg/ml), OFX (2.0 μg/ml), and KAN (30 μg/ml).

2.4. DNA Extraction

34 isolates were grown on solid media (Löwenstein-Jensen and OGAWA), and chromosomal DNA was extracted using the commercial kit method with MolecuTech REBA MTB-MDR 2011. The purified DNA pellet was stored at 4˚C until use.

2.5. Sequencing Method

Six loci were amplified by PCR: *katG*, *inhA*, and *ahpC* (INH); *rpoB* (RIF); *gyrA*

(OFX); and *rrs* (KAN) at Macrogen. Genetic site mutations in the corresponding resistance gene (*katG*, *inhA*, *ahpC*, *rpoB*, *gyrA*, and *rrs*) were performed using Macrogen molecular laboratory outsources. The primers are presented in **Table 1** [4] [13] [14]. The result of sequencing was then subjected to comparison and analysis.

2.6. Analysis

The sequencing data produced by the ABI 3730xl DNA analyzer were reviewed for confidence levels with an ABI sequence scanner, and chromatograms were analyzed for the presence or absence of mutations by comparison with published sequences of H37Rv. The data on clinical patients, resistant genes, genetic site mutation, and phenotype were compiled using the Excel 2010 database. Statistical analysis was performed using a statistical software package. The baseline characteristics of demographic data, treatment outcome and the genetic site mutation were presented using frequency and percentage. The associations between the demographic data, treatment outcome, resistant gene and MDR, Pre-XDR, and XDR were evaluated using exact probability tests. Univariable logistic regression was used to quantify the strength of the association between the demographic data, the gene mutation *with Mycobacterium tuberculosis* and the prevalence of Pre-XDR and XDR-TB among the MDR-TB patients.

Table 1. Primers used for sequencing.

Gene	Primer	Nucleotide sequencing (5' to 3')	Product size (pb)	Temp (˚C)	Reference
katG	MtkatGf MtkatGr	ACCCGAGGCTGCTCCGCTGG CAGCTCCCACTCGTAGCCGT	168	94˚C - 20 s 50˚C - 20 s 70 cycles 72˚C - 20 s	Afanas'ev MV, 2007
inhA	MtfabGf MtfabGr	GCCTCGCTGGCCCAGAAAGG CTCCGGATCCACGGTGGGT	320	94˚C - 20 s 56˚C - 20 s 70 cycles 72˚C - 20 s	Afanas'ev MV, 2007
ahpC	ahpC1 F ahpC2 R	GCCTGGGTGTTCGTCACTGGT CGCAACGTCGACTGGCTCATA	359	95˚C - 40 s 15 min (start) 94˚C - 40 s 30 cycles 57˚C - 40 s 1 min 72˚C - 40 s 15 min (final)	Valvatne H, 2009
rpoB	MtrpoBf MtrpoBr	GAGGCGATCACCGCAGAC GGTACGGCGTTTCGATGAAC	321	94˚C - 20 s 59˚C - 20 s 70 cycles 72˚C - 20 s	Afanas'ev MV, 2007
gyrA	gyrBA-3F gyrBA-3R	AAGAGCGCCACCGACATC CAGCATCTCCATCGCCAA	320	95˚C - 2 min (start) 95˚C - 30 cycles 1 min 65˚C - 1 min 72˚C - 1 min 72˚C - 10 min (final)	Liang L, 2012
rrs	16S-2F 16S-1R	CGTGGCCGTTTGTTTTGTC TGGTGCTCCTTAGAAAGGAGG		95˚C - 2 min (start) 94˚C - 35 cycles 1 min 60˚C - 1 min 68˚C - 2 min 68˚C - 10 min (final)	Liang L, 2012

3. Results

3.1. Characteristics of MDR-TB and Pre-XDR/XDR-TB

There was no statistically significant difference ($p < 0.05$) between the characteristic of MDR-TB and Pre-XDR/XDR-TB cases (Table 2).

Table 2. Characteristics of MDR and Pre-XDR/XDR-TB patients.

	n (%)		
Characteristics	MDR-TB N = 24	Pre-XDR or XDR-TB N-10	*p*-value
Gender			
Male	13 (54.2)	6 (60.0)	1.000
Female	11 (45.8)	4 (40.0)	
Age (year)			
0 - 20	1 (4.2)	0 (0.0)	
21 - 40	9 (37.5)	5 (50.0)	
41 - 60	11 (45.8)	4 (40.0)	0.098
>60	3 (12.5)	1 (10.0)	
Mean (SD)	44.6 (14.5)	43.3 (11.5)	
Nationality			
Thai	21 (87.5)	7 (70.0)	0.328
Non-Thai	3 (12.5)	3 (30.0)	
Treatment history			
New	14 (58.3)	4 (40.0)	0.457
Previous	10 (41.7)	6 (60.0)	
BMI			
<18.5	11 (45.8)	7 (70.0)	
≥18.5	13 (54.2)	3 (30.0)	0.270
Mean (SD)	18.9 (3.3)	17.0 (3.8)	
Chest x-ray			
Non-cavity	14 (58.3)	4 (40.0)	0.457
Cavity	10 (41.7)	6 (60.0)	
Sputum smear			
Negative	4 (16.7)	1 (10.0)	
AFB 1+	7 (29.2)	3 (30.0)	0.836
AFB 2+	5 (20.8)	1 (10.0)	
AFB 3+	8 (33.3)	5 (50.0)	
Comorbidity			
No	11 (91.7)	1 (10.0)	0.061
Yes	1 (8.3)	9 (90.0)	
Location			
ChiangMai	5 (20.8)	3 (30.0)	
ChiangRai	7 (29.2)	3 (30.0)	
Lampang	2 (8.3)	0 (0.0)	
Lamphun	1 (4.2)	0 (0.0)	1.000
Nan	3 (12.5)	1 (10.0)	
Phrae	5 (20.8)	2 (20.0)	
Phayao	1 (4.2)	1 (10.0)	

3.2. Treatment Patterns of MDR-TB and Pre-XDR/XDR-TB

The majority of treatments in of the MDR-TB and Pre-XDR/XDR-TB patients were similar that found combination directly observed and self-administered for therapy type, CAT V (I) for treatment pattern, during on treatment more than 24 months in Table 3. The majority of side effects were different in two groups that found minor side effect (75.0%) in MDR-TB patients but found major side effect (40.0%) in Pre-XDR or XDR-TB patients in Table 3.

3.3. Treatment Outcome of MDR-TB and Pre-XDR/XDR-TB

The treatment outcome resulting as "cure" was observed mainly in MDR-TB (50%). Cure/successful treatment was found 30% in Pre-XDR/XDR-TB group with defaulted (30%) and dead (30%) as shown in Table 4. However, it is found that 20% of deaths in Pre-XDR/XDR-TB patients occurred before the initiation of TB treatment.

Table 3. Treatment patterns of MDR and Pre-XDR/XDR-TB patients.

	n (%)		
Characteristics	MDR-TB N = 24	Pre-XDR or XDR-TB N-10	*p*-value
Therapy type			
No directly ibserved and self-adminstered	2 (8.3)	2 (20.0)	0.872
Directly observed only	3 (12.5)	1 (10.0)	
Self-administered only	4 (16.7)	2 (20.0)	
Combination directly ibserved and self-adminstered	15 (62.5)	5 (50.0)	
Treatment patterns			
No treatment	2 (8.3)	2 (20.0)	0.112
CAT IV (I)	13 (54.2)	4 (40.0)	
CAT IV (II)	9 (37.5)	2 (20.0)	
CAT V	0 (0.0)	1 (10.0)	
Only INH	0 (0.0)	1 (10.0)	
Period of treatment (months)			
<6.0	5 (20.8)	2 (20.0)	0.952
6.1 - 12.0	3 (12.5)	2 (20.0)	
12.0 - 24.0	7 (29.2)	3 (30.0)	
>24.0	9 (37.5)	3 (30.0)	
Min	0	2.3	
Max	48.3	51.1	
Mean (SD)	20.1 (1.4)	18.3 (14.4)	
Major side effects			
No	8 (33.3)	6 (60.0)	0.252
yes	16 (66.7)	4 (40.0)	
Minor side effect			
No	6 (25.0)	7 (70.0)	0.020
Yes	18 (75.0)	3 (30.0)	

Table 4. Treatment outcome for MDR and Pre-XDR/XDR-TB.

	n (%)		
Outcome of treatment	MDR-TB N = 24	Pre-XDR or XDR-TB N-10	*p*-value
Treatment outcome			0.697
Cure	12 (50.0)	3 (30.0)	
Complete	1 (4.2)	0 (0.0)	
Failure	1 (4.2)	1 (10.0)	
Dead	6 (25.0)	3 (30.0)	
Defaulted	4 (16.6)	3 (30.0)	

3.4. MDR-TB, and Pre-XDR/XDR-TB with Gene Mutation Codon

The analysis was conducted on 34 isolates of which 24 was MDR-TB, 9 Pre XDR and 1 XDR-TB. DNA sequencing was tested following six resistant genes: *katG*, *inhA*, *ahpC*, *rpoB*, *gyrA*, and *rrs*. The *katG*, *inhA*, *ahpC*, and *rpoB* indicated resistance to the first-line antibiotic treatment, while *gyrA* and *rrs* indicated resistance to the second-line antibiotic treatment. The distribution of MDR, Pre-XDR and XDR-TB by mutation site in *katG*, *inhA*, *ahpC*, *rpoB*, *gyrA*, and *rrs* gene can be seen in Table 5 and Table 6. The isoniazid (INH) resistant isolates had genetic site mutations within the *katG* gene, *inhA* gene, and *ahpC* gene, which had mutated in many codons (Table 5). The majority of the *katG* gene mutations in MDR-TB had a genetic site mutation in codon 315 (Table 5). There was no mutation in any *katG* codon of the 14 cases in MDR, Pre-XDR/XDR-TB (Table 5). There were two cases of isoniazid (INH) drug resistance that exhibited no mutation to any genetic site on the *katG*, *inhA*, and *ahpC* gene in the MDR-TB patients. Two cases found mutation only a *katG* gene in the Pre-XDR-TB patients in our study. Mutation of *rpoB* 445 codon was significantly found in Pre-XDR/XDR-TB isolates (50%) than in MDR-TB isolates (23.5%) with the p-value of 0.031 (Table 6).

3.5. Odds of Pre-XDR/XDR-TB by Clinical Profile and Loci of Gene Mutation

Our study found that Pre-XDR/XDR-TB patients significantly presented a mutation in the region of the *rpoB* gene at codon 445 (C445T) 20.6 times than the MDR-TB patients ($P = 0.026$) (Table 7). The results also showed that the prevalence *inhA* gene mutation at codon 114 (T114G) was significantly higher (8.1 times) in the Pre-XDR/XDR-TB patients than in the MDR-TB patients ($p = 0.034$) (Table 7). Also the data presented that minor side effect was significantly lower (0.14 times) in the Pre-XDR/XDR-TB patients than in the MDR-TB patients ($p = 0.020$) (Table 7).

The predictive markers in a logistic model (the mutation of the *inhA* gene at codon 114, the *rpoB* gene at codon 445, the *rrs* gene at codon 414 and minor

Table 5. Distribution of MDR-TB, and Pre-XDR-TB/XDR-TB by *katG*, *inhA*, and *ahpC* gene mutation codon.

Gene mutation codon	MDR-TB n (%)	Pre-XDR or XDR-TB n (%)	Total	*p*-value
katG				
No mutation	10 (41.7)	4 (40.0)	14 (41.2)	0.618
katG 315	10 (41.7)	2 (20.0)	12 (35.3)	0.211
katG 320	1 (4.2)	0 (0.0)	1 (2.9)	0.706
katG 300	0 (0.0)	1 (10.0)	1 (2.9)	0.294
katG 302	0 (0.0)	0 (0.0)	1 (2.9)	0.294
katG 314	1 (4.2)	0 (0.0)	1 (2.9)	0.706
katG 308	1 (4.2)	2 (20.0)	3 (8.8)	0.201
katG 299	0 (0.0)	1 (10.0)	1 (2.9)	0.294
katG 340	3 (12.5)	1 (10.0)	4 (11.8)	0.666
katG 343	5 (20.8)	2 (20.0)	7 (20.6)	0.670
katG 310	1 (4.2)	2 (20.0)	3 (8.8)	0.201
katG 312	1 (4.2)	3 (30.0)	4 (11.8)	0.067
InhA				
No mutation	5 (20.8)	2 (20.0)	7 (20.6)	0.670
inhA 14	8 (33.3)	3 (30.0)	11 (32.4)	0.591
inhA 25	1 (4.2)	0 (0.0)	1 (2.9)	0.706
inhA 78	5 (20.8)	3 (30.0)	8 (23.5)	0.435
inhA 81	5 (20.8)	2 (20.0)	7 (20.6)	0.670
inhA 84	6 (25.0)	3 (30.0)	9 (26.5)	0.538
inhA 86	6 (25.0)	2 (20.0)	8 (25.5)	0.565
inhA 94	3 (12.5)	0 (0.0)	3 (8.8)	0.338
inhA 114	5 (20.8)	6 (60.0)	11 (32.4)	0.036
ahpC				
No mutation	14 (58.3)	7 (70.0)	21 (61.8)	0.406
ahpC 10	2 (8.3)	2 (20.0)	4 (11.8)	0.334
ahpC 12	2 (8.3)	2 (20.0)	4 (11.8)	0.334
ahpC 20	3 (12.5)	1 (10.0)	4 (11.8)	0.666
ahpC 22	2 (8.3)	1 (10.0)	3 (8.8)	0.662
ahpC 75	6 (25.0)	1 (10.0)	7 (20.6)	0.315
ahpC 76	5 (20.8)	1 (10.0)	6 (17.7)	0.416

Table 6. Distribution of MDR-TB, and Pre-XDR/XDR-TB by *rpoB*, *gyrA* and *rrs* gene mutation codon.

Gene mutation codon	MDR-TB n (%)	Pre-XDR or XDR-TB n (%)	Total	*p*-value
rpoB				
No mutation	4 (16.7)	2 (20.0)	6 (17.7)	0.584
rpoB 445	3 (12.5)	5 (50.0)	8 (23.5)	0.031
rpoB 450	5 (20.8)	1 (10.0)	6 (17.7)	0.416
rpoB 464	6 (25.0)	0 (0.0)	6 (17.7)	0.100
rpoB 483	2 (8.3)	2 (20.0)	4 (11.8)	0.334
rpoB 490	5 (20.8)	2 (20.0)	7 (20.6)	0.670
rpoB 493	2 (8.3)	2 (20.0)	4 (11.8)	0.334
rpoB 507	7 (29.2)	2 (20.0)	9 (26.5)	0.462
rpoB 508	8 (33.3)	1 (10.0)	9 (26.5)	0.165

Continued

gyrA				
No mutation	5 (20.8)	2 (20.0)	7 (20.6)	0.670
gyrA 21	1 (4.2)	0 (0.0)	1 (2.9)	0.706
gyrA 70	2 (8.3)	0 (0.0)	2 (5.9)	0.492
gyrA 87	17 (70.8)	6 (60.0)	23 (67.7)	0.409
gyrA 102	1 (4.2)	0 (0.0)	1 (2.9)	0.706
gyrA 162	10 (41.7)	5 (50.0)	15 (44.1)	0.471
gyrA 187	8 (33.3)	4 (40.0)	12 (35.3)	0.502
rrs				
No mutation	16 (66.7)	3 (30.0)	19 (60.2)	0.057
rrs 223	2 (8.3)	3 (30.0)	5 (14.7)	0.138
rrs 241	1 (4.2)	1 (10.0)	2 (5.9)	0.508
rrs 408	5 (20.8)	4 (40.0)	9 (26.5)	0.230
rrs 414	2 (8.3)	4 (40.0)	6 (17.7)	0.048
rrs 512	1 (4.2)	1 (10.0)	5 (5.9)	0.508

Table 7. Odds of Pre-XDR/XDR-TB by loci of gene mutation.

Gene	Loci of mutations	Odd ratio	95% CI	*p*-value
inhA	No mutation	1.00	Reference	-
	inhA 14	0.66	0.10 - 4.55	0.676
	inhA 81	3.00	0.14 - 65.52	0.484
	inhA 84	1.23	0.09 - 17.00	0.876
	inhA 86	0.60	0.04 - 8.19	0.702
	inhA 114	8.12	1.17 - 56.10	0.034
ahpC	No mutation	1.00	Reference	-
	ahpC 10	1.88	0.17 - 20.11	0.602
	ahpC 12	1.88	0.17 - 20.11	0.602
	ahpC 76	0.51	0.05 - 5.31	0.577
rpoB	No mutation	1.00	Reference	-
	rpoB 445	20.64	1.44 - 295.42	0.026
	rpoB 450	0.54	0.03 - 8.35	0.659
	rpoB 483	1.51	0.08 - 29.17	0.783
	rpoB 490	0.23	0.01 - 4.59	0.336
	rpoB 507	3.53	0.30 - 41.70	0.317
	rpoB 508	12.23	0.20 - 757.35	0.234
rrs	No mutation	1.00	Reference	-
	rrs 223	1.85	0.15 - 22.38	0.628
	rrs 241	0.70	0.01 - 32.78	0.856
	rrs 408	2.17	0.28 - 16.60	0.456
	rrs 414	6.90	0.77 - 61.89	0.084
	rrs 512	3.68	0.17 - 77.73	0.402
Minor side effect	No minor side effect	1.00	Reference	-
	Minor side effect	0.14	0.15 - 22.38	0.020

side effect can be explained 89.6% the probability of Pre-XDR/XDR-TB among MDR-TB (Figure 2).

4. Discussion

The predictors of Pre-XDR/XDR-TB from MDR-TB that will be useful for early treatment need to be identified from the genetic mutation marker. Mutations in

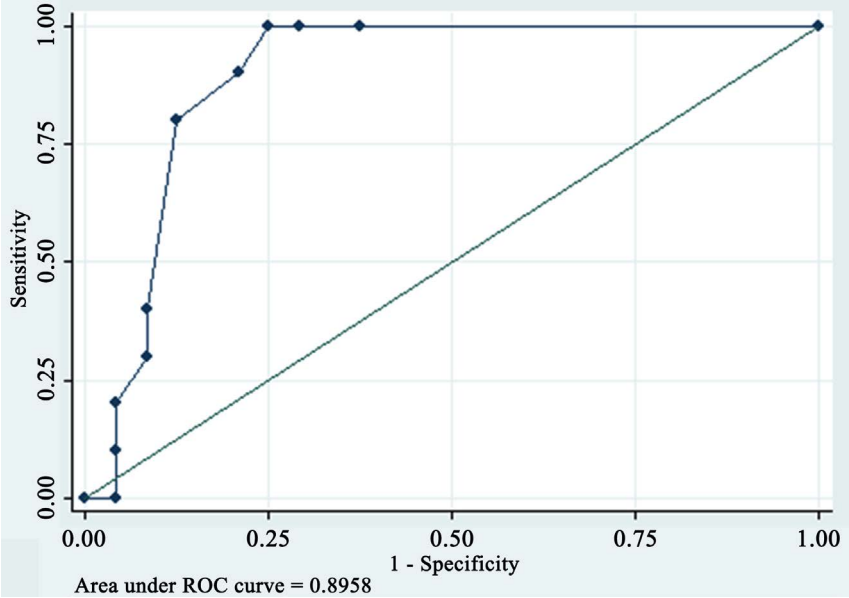

Figure 2. Receiver operating characteristic (ROC) curve of predictive markers of Pre-XDR/XDR-TB among MDR-TB.

the selected genes of *M. tuberculosis* have been used as markers for anti-TB drug resistance. Our results found that DST phenotypic resistance correlated with resistant genes, isoniazid resistance and *katG*, *inhA*, *ahpC*; and rifampicin resistance and *rpoB*.

Gene mutation site in MDR-TB and Pre-XDR/XDR-TB patents: The *rpoB* gene mutation was a significant factor in terms of increasing the severity of MDR-TB, which may lead to the diagnosis (prediction) of Pre-XDR-TB and XDR-TB in patients. Previous study showed that 31.2% of the primary MDR-TB patients in China had S531L *rpoB* mutation [15]. Wang Sheng Fen study further showed that the combination of mutations in *gyrA*, *rrs*, and *tlyA* could predict Pre-XDR-TB with 68.9% sensitivity and XDR-TB with 65.9% sensitivity and 100% specificity [16].

4.1. *InhA* 114 among Pre-XDR/XDR-TB Patients and MDR-TB Patients

Our study showed that the *inhA* gene mutation position at 114 (T114G) and the *rpoB* gene mutation position at 445 (C445T) maybe used as a tool to predict the Pre-XDR/XDR-TB patients. The mutation of T114G or C445T was more likely to be associated with the development to Pre-XDR-TB and XDR-TB among MDR-TB, with the chance of 8.1 and 20.6 times, respectively. In our study, gene mutation in *inhA* 114 was detected in 82.6% (19/23) of the MDR and in 17.4% (4/23) of the Pre-XDR or XDR-TB strains. There have been no previous reports of *inhA* 114 mutation in MDR-TB and XDR-TB strains; however this could be a case of silent mutation. Mutations of *inhA* are also commonly found at (−15) [17] [18] [19] [20] among the *Mycobacterium tuberculosis* drug resistant strains that can be found among TB and MDR-TB patients.

4.2. *RpoB* 445 among Pre-XDR or XDR-TB Patients or MDR-TB Patients

Many studies have documented that *rpoB* 445 is very specific to rifampicin resistance, which has been used to detect MDR-TB [21] [22] [23]. In our study, gene mutation in *rpoB* was detected in 85.3% (29/34) of the MDR and XDR-TB strains and was more likely to be found in Pre-XDR and XDR-TB patients by about 20 times when compared with the MDR-TB patients. One study showed that *rpoB* 445 was a very strong factor in predicting rifampicin resistance [24]. A previous study in Swaziland showed *rpoB* 445 mutation in MDR-TB patients (79.17%) [22]. The *rpoB* 445 mutation was also found during the outbreak of MDR-TB in Argentina in 1973 [23]. Previous studies have shown that *rpoB*445 could predict MDR with high specificity but low sensitivity [19].

4.3. Minor Side Effect among Pre-XDR/XDR-TB Patients or MDR-TB Patients

The attention paid for treatment of ADR with minimum modification of treatment regimen that was increased cure rate [25]. Also the previous study in MDR-TB without co-infection with HIV showed ADR was not effect to stop treatment [26]. Minor side effects appeared to have little impact on treatment completion and the conversion to Pre-XDR/XDR-TB because patients tended to visit healthcare providers more often to discuss their side effect concerns, resulting in better continuation of care and treatment which indirectly lowering the conversion to Pre-XDR/XDR-TB.

5. Conclusion

In conclusion, our study has found that presence of mutations in *inhA* 114 and *rpoB* 445 could be an indicator for Pre-XDR and XDR-TB strains among MDR-TB patients in northern Thailand. Prospective results should be done before applying these mutations as markers for Pre-XDR and XDR-TB in this population.

6. Limitations of This Study

The limitation of this study was that it was a retrospective study where the evaluation was carried out using only one-fifth isolates that could be subcultured from a total of 161 isolates. The sample size was rather limited. Generalization from this study should be made with caution.

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