Drug resistance patterns of Mycobacterium tuberculosis complex and risk factors associated with multidrug-resistant tuberculosis in the upper southern part of Thailand

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ABSTRACT

Background: this study aimed to assess the drug resistant pattern of *Mycobacterium tuberculosis* complex (MTBC) and the risk factors associated to multidrug-resistant tuberculosis cases (MDR-TB) in upper part of southern Thailand.
Methods: a total of 3238 TB cases was retrieved from a database of the office of prevention and control disease region 11. Only 1008 cases were confirmed by culture growth for *Mycobacterium tuberculosis* and drug-susceptibility testing (DST) during a period of 4 years (January 2013 to December 2016). The risk factors, including gender, age group, residence place, and history of treatment were analysed using multivariate logistic regression to predict the MDR-TB cases.
Results: among 1008 TB cases included in study, 77.4% of them were males, 31.5% lived in rural area with median age of 45.0 years (IQR = 23.0), 27.6% were retreatment for tuberculosis, 25.9%, 10.8%, 3.0%, 10.7% and 9.1 were determined to be resistant to isoniazid, rifampicin, ethambutol, streptomycin and MDR-TB, respectively. Adjusted odds ratios (95% confidence interval) of MDR-TB were 5.4 (2.68-11.03), and 4.2 (2.10, 8.45) for retreatment patients, and on treatment patients, respectively.
Conclusions: drug resistance tuberculosis is considerable problem in upper part of southern Thailand. Major risk factors involved previous history of TB treatment. Thus, it emphasizes on patients who had a history of previous TB treatment.

Key words: tuberculosis, MDR-TB, Multidrug-resistant, Drug susceptibility test

INTRODUCTION

Multi-drug resistant tuberculosis (MDR-TB) is a serious problem where the bacterium is resistant to at least two of the most powerful first-line anti-TB drugs such as Rifampicin (RIF) and Isoniazid (INH) with or without any other drug. In 2016, of the estimated 600000 had MDR/RR-TB. A total of 153119 patients were enrolled and started on MDR/RR-TB treatment. Globally, data show an average success rate of only 54% for treated MDR/RR-TB patients, whereas 8% the treatment failed, 16% died, 15% were lost to follow-up, and 7% had no outcome information [1]. The high rate MDR/RR-TB found in South-East Asia where in certain countries more than 13% of previously treated and more than 2.8% of new TB cases. Almost half (47%) of these cases were in India, China, and the Russian Federation. In addition, as many as 6.2% of MDR-TB case were extensively drug-resistant TB (XDR-TB) [2].

Thailand is one of the 30 high MDR/RR-TB burden countries in the world. WHO estimated incidence case in 2015-2016 equal 6.6 and 6.8 per 100000 populations, respectively. Furthermore, in 2015, the new cases of MDR/RR-TB and the previously treated TB cases are 2.2% and 24%, respectively which the number not change in 2016 [2, 3]. This situation presented the problem of MDR/RR-TB are still remain in Thailand. Therefore, the management of patients with MDR-TB should be applied with the drug-susceptibility testing (DST) to identify them. However, DST was be used only in some hospitals in Thailand: the Provincial Hospitals, University Hospitals, Tuberculosis Center, and the office of prevention and control disease region 11 (DPC11).

The DPC11 in Nakhon Si Thammarat province which is only one the reference laboratory centers for tuberculosis, particularly MDR-TB in upper part of southern, Thailand. The DPC11 provides *mycobacterium tuberculosis* (MTB) culture and DST to inpatient and outpatients from health facilities in seven provinces of southern Thailand, including Chumphon, Krabi, Phuket, Phang Nga, Surat Thani, Nakhon Si Thammarat, and Ranong. However, the data of drug resistance pattern with DST was done, but its lack to identify risk factors and drug resistant pattern of *Mycobacterium tuberculosis* complex from routine laboratory testing data. Therefore, we conducted a retrospective study to determine the frequency of pattern of drug resistance to first-line anti-TB drugs and risk factors for MDR-TB in upper part of southern Thailand.

METHODS

Definition

Monoresistance: resistance to one first-line anti-TB drug only.

Polydrug resistance: resistance to more than one first-line anti-TB drug, other than both isoniazid and rifampicin.

Multidrug resistance: resistance to at least both isoniazid and rifampicin.

Any resistance: resistance to any of the anti-TB drugs.

On treatment: TB patients who, while on treatment, are sputum smear-positive at month three or later during the course of treatment are at elevated risk for drug-resistant TB.

Pre-treatment: The new TB patients in close contacts with MDR-TB patients in the past or household contact.

Re-treatment: Previously treated patients such as after failure of retreatment regimen with first-line drugs, after failure of first treatment with first-line drugs, relapse, and after loss to follow-up

Study design, area

A cross-sectional study using routinely collected of the laboratory data of patients with TB, diagnosed between January 1, 2013 and December 31, 2016. A total of 3238 specimens were collected from TB patients at the office of prevention and control disease region 11 (DPC11).

Inclusion and exclusion criteria

We included TB patient with sputum culture was defined as *Mycobacterium tuberculosis* colony growth and excluded TB patients with extra-pulmonary tuberculosis, Prisoner and TB&HIV co-infection.

Processing of sputum specimens

Microbiological methods: microscopy

Screening for acid-fast bacilli (AFB) by conventional sputum smear microscopy of direct smears of sputum is the first line test used for pulmonary tuberculosis (PTB) laboratory diagnosis. Positive slides were further confirmed by staining with Kinyoun modification of the ZiehlNeelsen stain [4-6].

Microbiological methods: isolation of M.tuberculosis

Mycobacterial culture was performed on both liquid and solid media. Sediments were cultured at 37 degrees using Lowenstein-Jensen (LJ) medium and ¨BACTECTM MGITTM (Mycobacteria Growth Indicator Tube; BD, Sparks, MD, USA) according to the WHO recommendation [7]. For the LJ slant, 0.1 ml of concentrated specimen was inoculated and incubated for 8 weeks [8]. MGIT vials were inoculated with 0.5 ml of specimen and incubated at 37 degrees for 42 days maximum [9]. All positive culture results were confirmed by using BD MGITTM TBc Identification Test and SD BIOLINE TB ag MPT64 Rapid 64 [10, 11]

Microbiological methods: drug susceptibility testing

Phenotypic DST of the isolates against rifampicin, isoniazid, ethambutol and streptomycin was done using the BACTEC MGIT 960 indirect proportion method. The drugs were used at concentrations of 1.0 µg/ml for rifampicin, 0.1 µg/ml for isoniazid, 5.0 µg/ml for ethambutol and 1.0 µg/ml for streptomycin [12]. Genotypic DST (Molecular DST) of the isolates against rifampicin and isoniazid was done using line probe assays (LPA) [13].

Data analysis

Data were entered using Epidata and exported to STATA version 10 for analysis. Data completeness and consistency were checked by running frequencies of each variable. Median and interquartile range (IQR) were presented for age distributions. Pearson Chi-square statistics test were used to compare categorical variables. All variables were examined in a univariate analysis and all variables with a p-value ≤ 0.2 were included in a multivariate logistic regression analysis. Multivariate analysis was performed by binary logistic regression using backwards analysis (variables were included and excluded from the model using a cut-off p-value of, 0.05). We assessed the model fit using Hosmer-Lemeshow goodness-of-fit test, p-value > 0.05 is considered as a good fit.

RESULTS

Baseline characteristics

Out of 3238 TB case, 1314 (40.6%) were growth, 1543 (47.7%) no growth, 103 (3.2%) found contaminated, 208 (6.4%) not available and culture could not be performed on 70 (2.2%) cases because of poor quality of specimens. Among 1314 culture specimens and detection of growth, 151 were found to be Non-tuberculous mycobacteria (NTM)

TABLE 1. Drug resistance pattern in Mycobacterium tuberculosis complex (n = 1008)

Drug Resistance Pattern	On treatment (n = 344)	Pre-treatment (n = 386)	Re-treatment (n = 278)	All case (n = 1008)
	n (%)	n (%)	n (%)	n (%)
Drug-sensitive tuberculosis	217 (63.1)	307 (79.5)	166 (59.7)	690 (68.5)
Any Resistance	127 (36.9)	79 (20.5)	112 (40.3)	217 (63.1)
INH	108 (31.4)	61 (15.8)	92 (33.1)	261 (25.9)
RIF	45 (13.1)	13 (3.4)	51 (18.3)	109 (10.8)
EMB	12 (3.5)	8 (2.1)	10 (3.6)	30 (3.0)
STM	36 (10.5)	35 (9.1)	37 (13.3)	108 (10.7)
Mono Resistance (n = 180)	77 (22.4)	49 (12.7)	54 (19.4)	180 (17.9)
INH	58 (16.9)	32 (8.3)	35 (12.6)	125 (12.4)
RIF	6 (1.7)	2 (0.5)	8 (2.9)	16 (1.6)
EMB	2 (0.6)	2 (0.5)	3 (1.1)	7 (0.7)
STM	11 (3.2)	13 (3.4)	8 (2.9)	32 (3.2)
Poly Resistance (n = 46)	11 (3.2)	19 (4.9)	16 (5.8)	46 (4.6)
INH + EMB	3 (0.9)	3 (0.8)	2 (0.7)	8 (0.8)
INH+ STM	6 (1.7)	14 (3.6)	10 (3.6)	30 (3.0)
RIF+ STM	0 (0.0)	0 (0.0)	1 (0.4)	1 (0.1)
EMB+ STM	0 (0.0)	1 (0.3)	0 (0.0)	1 (0.1)
INH + EMB+ STM	2 (0.6)	1 (0.3)	3 (1.1)	6 (0.6)
MDR-TB (n = 92)	39 (11.3)	11 (2.8)	42 (15.1)	92 (9.1)
INH+ RIF	22 (6.4)	5 (1.3)	26 (9.4)	53 (5.3)
INH+ RIF+ EMB	0 (0.0)	0 (0.0)	1 (0.4)	1 (0.1)
INH+ RIF+ STM	12 (3.5)	5 (1.3)	14 (5.0)	31 (3.1)
INH+ RIF+ EMB+ STM	5 (1.5)	1 (0.3)	1 (0.4)	7 (0.7)

INH: Isoniazid, RIF: Rifampicin, STM: Streptomycin, EMB: Ethambutol

TABLE 2. Association between patient's demographics and drug resistance (n = 1008)

Variables No	Isoniazid		Rifampicin		Ethambutol		Streptomycin		MDR-TB	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	
Total	747 (74.1%)	261 (25.9%)	899 (89.2%)	109 (10.8%)	978 (97.0%)	30 (3.0%)	900 (89.3%)	108 (10.7%)	916 (90.9%)	92 (9.1%)
Gender										
Female	166 (72.8%)	62 (27.2%)	197 (86.4%)	31 (13.6%)	222 (97.4%)	6 (2.6%)	198 (86.8%)	30 (13.2%)	204 (89.5%)	24 (10.5%)
Male	581 (74.5%)	199 (25.5%)	702 (90.0%)	78 (10.0%)	756 (96.9%)	24 (3.1%)	702 (90.0%)	78 (10.0%)	712 (91.3%)	68 (8.7%)
p-value	0.610		0.125		0.728		0.175		0.405	
Age group (years)										
< 60	586 (74.4%)	202 (25.6%)	693 (87.9%)	95 (12.1%)	765 (97.1%)	23 (2.9%)	698 (88.6%)	90 (11.4%)	709 (90.0%)	79 (10.0%)
≥ 60	161 (73.2%)	59 (26.8%)	206 (93.6%)	14 (6.4%)	213 (96.8%)	7 (3.2%)	202 (91.8%)	18 (8.2%)	207 (94.1%)	13 (5.9%)
p-value	0.723		0.018 *		0.839		0.170		0.065	
Residence place										
Rural	224 (70.7%)	93 (29.3%)	265 (83.6%)	52 (16.4%)	304 (95.9%)	13 (4.1%)	281 (88.6%)	36 (11.4%)	274 (86.4%)	43 (13.6%)
Urban	532 (75.7%)	168 (24.3%)	634 (91.8%)	57 (8.2%)	674 (97.5%)	17 (2.5%)	619 (89.6%)	72 (10.4%)	642 (92.9%)	49 (7.1%)
p-value	0.091		< 0.001*		0.155		0.655		0.001*	
TB treatment History										
Retreatment	186 (66.9%)	92 (33.1%)	227 (81.7%)	51 (18.3%)	268 (96.4%)	10 (3.6%)	241 (86.7%)	37 (13.3%)	236 (84.9%)	42 (15.1%)
On treatment	236 (68.6%)	108 (31.4%)	299 (86.9%)	45 (13.1%)	332 (96.5%)	12 (3.5%)	308 (89.5%)	36 (10.5%)	305 (88.7%)	39 (11.3%)
Pre-treatment	325 (84.2%)	61 (15.8%)	373 (96.6%)	13 (3.4%)	378 (97.9%)	8 (2.1%)	351 (90.9%)	35 (9.1%)	375 (97.2%)	11 (2.8%)
p-value	< 0.001*		< 0.001*		0.412		0.215		< 0.001*	

Values are presented as number (%).
**p-values < 0.05 of the chi-square test.*

and 1163 found *Mycobacterium tuberculosis*. Culture no growth specimens, contaminated, waiting, Non-tuberculous mycobacteria and those on which DST could not be performed were excluded from the study. The DST could not be applied on 155 cases. Thus 1008 patients were included in the final analysis process (Fig 1). The median ages of patients were 45.0 (IQR = 23.0) years, 228 (22.6%) female, 780 (77.4%) male, 691 (68.6%) urban residency whereas 317 (31.4%) were rural, 344 (34.1%) on treatment, 386 (38.3%) were pre-treatment and 278 (27.6%) re-treatment (Table 2).

Patterns of drug resistance

The rate of any resistance to ethambutol (EMB), streptomycin (STM), isoniazid (INH) and rifampicin (RIF) were 30/1008 (3.0%), 108/1008 (10.7%), 261/1008 (25.9%), and 109/1008 (10.8 %), respectively. Moreover, among patients who resistant with rifampicin or isoniazid drug have chance to be MDR-TB 92/1008 (9.1%). Furthermore, 180/1008 (17.9%) were mono drug resistant, and 46/1008 (4.6%) poly drug resistant (Table 1).

Factors associated with drug resistance

Upon Chi square analysis statistical significant association was observed between TB treatment history and isoniazid resistance TB ($p < 0.001$). Age group ($p = 0.016$), Residence place ($p < 0.001$), and TB treatment history ($p < 0.001$) showed a significant association with rifampicin resistance TB while gender was not significantly associated with rifampicin resistance TB. Furthermore, gender ($p = 0.124$), age group ($p = 0.839$), residence place ($p = 0.155$), and TB treatment history ($p = 0.412$) were not significantly associated with Ethambutol resistance TB. In addition, gender ($p = 0.175$), Age group ($p = 0.170$), Residence place ($p = 0.655$), and TB treatment

TABLE 3. Multivariate logistic regression result of risk factors for development of Rifampicin resistance tuberculosis (RR-TB)

Variables	Rifampicin resistance		Univariate		Multivariate	
	No	Yes	cOR (95% CI)	p-value	aOR (95% CI)	p-value
Gender						
Female	197 (86.4%)	31 (13.6%)	1.4 (0.91, 2.21)	0.125	1.6 (1.01, 2.54)	0.047
Male	702 (90.0%)	78 (10.0%)	1		1	
Age group (years)						
< 60	693 (87.9%)	95 (12.1%)	2.0 (1.13, 3.61)	0.018	2.0 (1.12, 3.67)	0.019
≥ 60	206 (93.6%)	14 (6.4%)	1		1	
Residence place						
Rural	265 (83.6%)	52 (16.4%)	2.2 (1.46, 3.26)	< 0.001	1.4 (0.94, 2.20)	0.095
Urban	634 (91.8%)	57 (8.2%)	1		1	
TB treatment History						
Retreatment	227 (81.7%)	51 (18.3%)	6.4 (2.29, 8.15)	< 0.001	5.7 (2.98, 11.05)	< 0.001
On treatment	299 (86.9%)	45 (13.1%)	4.3 (3.43, 12.11)		4.2 (2.12, 7.98)	
Pre-treatment	373 (96.6%)	13 (3.4%)	1		1	

cOR Crude Odds Ratio, aOR Adjusted Odds Ratio

history (p = 0.215) were not significantly associated with streptomycin resistance TB. Finally, Residence place (p = 0.001) and TB treatment history (p < 0.001) showed a significant association with MDR-TB, whereas gender (p = 0.405) and Age group (p = 0.061) were not significantly associated with the development of MDR-TB (Table 2).

The multivariable logistic regression model identified gender (aOR=1.6; 95% CI: 1.01, 2.54), age group < 60 years (aOR=2.0; 95% CI: 1.12, 3.67), retreatment patients (aOR=5.7; 95% CI: 2.98, 11.05) and on treatment patients (aOR=4.2; 95% CI: 2.12, 7.98) to be associated with rifampicin resistance While residence place of TB case were not significantly associated with rifampicin resistance (Table 3). Furthermore, retreatment patients (aOR=5.4; 95% CI: 2.68, 11.03) and on treatment patients (aOR=4.2; 95% CI: 2.10, 8.45) were significantly associated with the development of MDR-TB, whereas gender, age group, and residence place were not significantly associated with the development of MDR-TB (Table 4).

DISCUSSION

In this study, drug resistance pattern of *mycobacterium tuberculosis* complex and associated factor were evaluated. Here we presented the results of a large number of TB sample collected from health facilities in seven provinces of upper part of southern Thailand.

The highest proportion of any drug resistance was observed to isoniazid 25.9%. This is comparable with the study conducted in Pakistan which showed 37.1% [14] and recent report from study in Ramathibodi Hospital, Thailand; 6.9% [15]. However, our finding was higher than that of previous studies in Ethiopia; 48.7% [16]. The higher prevalence of isoniazid resistance has also important implications. Isoniazid is the cornerstone drug used throughout the course of non-MDR-TB treatment. It is also the drug of choice for chemoprophylaxis of TB in developing countries for treating latent TB infection. Loss of the effectiveness of this drug compromises both the preventive therapy and treatment of TB disease. Moreover, it is predictor for MDR-TB in the future since MDR-TB often was developed from initial isoniazid mono-resistant strains [17].

The second highest any resistance was against rifampicin 10.8%. This is in agreement with the study in Hangzhou, China 10.2% [18]. The higher rate of rifampicin resistance might be due to its adverse effects such as vomiting, influenza like syndrome, fever, cut nausea, gastrointestinal, hepatitis, jaundice, and acute renal failure which could result in patient non-adherence and hence may lead to the selection of resistant strains [19, 20]. In this study, there were only sixteen cases with rifampicin as a mono resistance. The low proportion (1.6 %) of non-MDR rifampicin resistance in this study supports the use of rifampicin resistance as surrogate marker for MDR-TB.

The rate of streptomycin resistance was 10.7%. This rate was higher when compared with previous studies in Thailand where streptomycin resistance accounted for 6.2% [21]. However, the result is lower than that of recent study in northern Thailand 17.5 [22]. High any resistance to streptomycin may be because of its early introduction, its common use for treatment of other bacterial infections and inadequate treatment due to poor compliance by patients [23].

The proportion of any ethambutol resistance was 3.0 %.

TABLE 4. Multivariate logistic regression result of risk factors for development of MDR-TB

Variables	MDR-TB		Univariate		Multivariate	
	No	Yes	cOR (95% CI)	p-value	aOR (95% CI)	p-value
Gender						
Female	204 (89.5%)	68 (8.7%)	1.2 (0.75, 2.01)	0.405	1.4 (0.82, 2.26)	0.228
Male	712 (91.3%)	24 (10.5%)	1		1	
Age group (years)						
< 60	709 (90.0%)	79(10.0%)	1.8 (0.97, 3.26)	0.064	1.8 (0.96, 3.30)	0.067
≥ 60	207 (94.1%)	13 (5.9%)	1		1	
Residence place						
Rural	274 (86.4%)	43 (13.6%)	2.1 (1.33, 3.17)	< 0.001	1.4 (0.86, 2.15)	0.184
Urban	642 (92.9%)	49 (7.1%)	1		1	
TB treatment History						
Retreatment	236 (84.9%)	42 (15.1%)	6.1 (3.06, 12.02)	< 0.001	5.4 (2.68, 11.03)	< 0.001
On treatment	305 (88.7%)	39 (11.3%)	4.4 (2.20, 8.66)		4.2 (2.10, 8.45)	
Pre-treatment	375 (97.2%)	11 (2.8%)	1		1	

cOR Crude Odds Ratio, aOR Adjusted Odds Ratio

This rate was higher when compared with previous studies in Ubonratchatani, Thailand where ethambutol resistance accounted for 2.2% [24] and report from study at the Central Chest Hospital, Thailand; 1.5% [25]. However, WHO recommendation used ethambutol for the shorter MDR-TB regimen. Therefore, ethambutol resistance will be measure via reliable test before shorter MDR-TB treatment [26]. The high rate of ethambutol resistance would challenge its inclusion in MDR-TB therapy as this may lead to unintentional incorrect therapy [27]. Thus, further study should explore the level of ethambutol resistance specifically in MDR-TB isolates. This can help in developing regional standardized second line treatment regimen for MDR-TB cases.

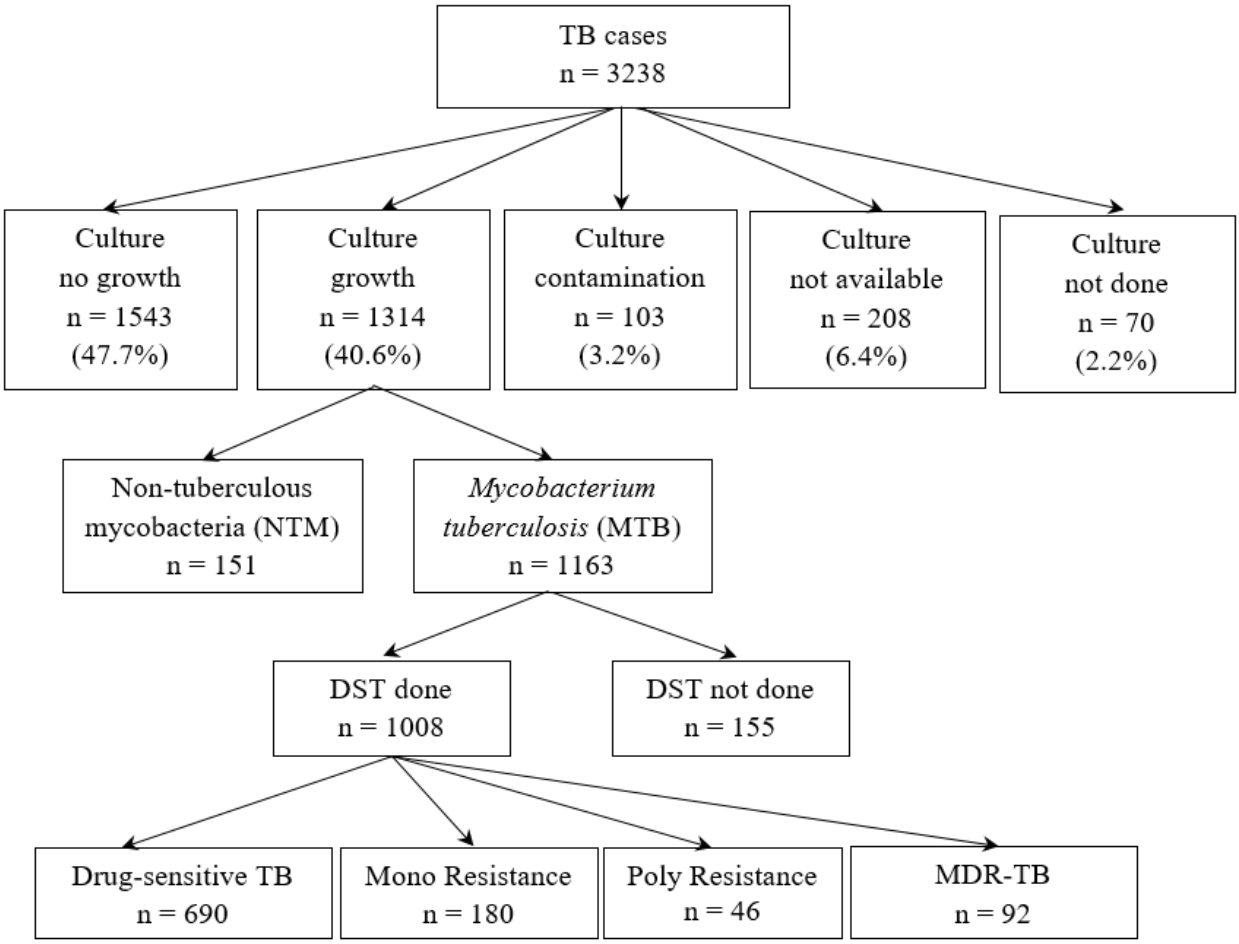
Among the resistant case in this study, the proportion of MDR-TB in retreatment, on treatment, and pre-treatment patients were 15.1%, 11.3%, and 2.8% respectively ,which is similar to previous studies [28-30]. MDR-TB reported to be different in different geographical areas. The prevalence of MDR-TB in new and retreatment case in China has been reported as 3% and 22% respectively [31], from Iran 9.4% and 90.6% and in another study 3.8 and 25.4 in new and retreatment cases, respectively [32, 33]. The study in Italy Ferrara et al. reported the overall MDR-TB as 127 case, in newly diagnosed was 41.0% and in previous diagnosed patients was 59% [34]. These difference may be due to different levels of health care delivery system in various countries, TB Control Program, living standards, and socio-economic factors. The resistance in retreatment cases is indicator of poor compliance, lack of treatment supervision and ineffective TB Control Program whereas in new cases is due to the transmission of disease with resistant bacilli [35].

The original drug-resistant TB is a result of chromosomal alterations due to mutations. There are several factors related to TB control program that have a significant impact on the increasing and transmission of drug-resistant TB [36]. In the other studies age, gender, prior TB therapy, residence place, duration of illness, abnormal chest radiological finding and sputum AFB positive showed significant association with any drug resistance [9, 37]. In this study, residence place, retreatment TB case and age group showed statistical significance in univariate analysis association with any drug resistance. Overall multivariate analysis showed that being retreatment TB cases were found to have statistically significant association with MDR-TB and rifampicin resistance. This is consistent with the studies in Ethiopia [38], Norway [39], and China [40]. Our result showed that more than fifteen percent of treatment failures were identified as MDR-TB. Majority of these cases were from category I treatment failures. This suggests the importance of test for culture and DST before initial treatment of MDR-TB.

High rates of MDR-TB among treatment failures (18.7%) can be influenced by the acquisition of resistance in the intensive and continuation phases of treatment or the rate of primary MDR-TB infection [41]. Therefore, most possible reason for higher rate of MDR-TB in our study is acquisition of drug resistance during the intensive or/and continuation phases of treatment. This may provide clue for the importance of evaluation of currently available TB control programs on proper usage of drugs. Moreover, it supports the necessity of looking in to the adherence of patients to full course of chemotherapy.

Our study suggests that patients from rural area were more likely to harbor drug resistant TB bacilli because of relatively lower access to laboratory for culture and drug

FIGURE 1. Flow chart of specimens' over all processing for investigating MDR-TB isolates.



susceptibility testing (DST) with first-line drug (rifampicin, isoniazid, ethambutol, pyrazinamide, streptomycin). This recommendation is for DST at the start of therapy for all previously treated patients [42]. Findings and treating MDR-TB in previously treated patients will help to improve better outcomes among these patients.

This study has several limitations. First, the study was conducted only in the upper part of southern Thailand under the responsibility of the office of prevention and control disease region 11. Therefore, the data may not be representation of the population at large. Second, laboratory data did not report behavioral risk factors such as alcohol, smoking, and diabetes also risk factors did not involve for data analysis. Finally, drug sensitivity testing for second-line anti-TB drugs did not perform for all MDR-TB patients. It is an institution based study. Despite this limitation, this study provided the first information on TB drug resistance among previously treated cases in the study setting. This can be used for better planning of TB management and tackling further increase in the level of MDR-TB.

In conclusion, High prevalence of isoniazid resistance and MDR-TB were detected among retreatment TB cases in seven provinces in upper part of southern Thailand. The proportion of MDR-TB was significantly higher among patients with the history of treatment failures. TB patients with history of treatment failures should be referred for culture and DST.

Ethics

Ethical approval was obtained from the ethical review board at the Kasetsart University in Thailand.

Competing Interests

The authors have no conflicts of interest to declare for this study.

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