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Biopsychosocial factors associated with Extensively Drug-Resistant Tuberculosis in Thailand

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Biopsychosocial factors associated with Extensively Drug-Resistant Tuberculosis in Thailand

A dissertation submitted in partial satisfaction of the

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in Epidemiology

by

Kaewalee Soontornmon

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ABSTRACT OF THE DISSERTATION

Biopsychosocial factors associated with Extensively Drug-Resistant Tuberculosis in Thailand

by

Kaewalee Soontornmon

Doctor of Philosophy in Epidemiology

University of California, Los Angeles, 2020

Professor Sung-Jae Lee, Co-Chair

Professor Roger Detels, Co-Chair

Background: Tuberculosis (TB) is one of the top ten causes of death by a single infectious agent. Thailand is also included in the list of high-burden countries of TB, TB/HIV, and Multidrug-Resistant TB (MDR-TB) defined by the World Health Organization. Extensively Drug-Resistant TB (XDR-TB) is a type of MDR TB, defined as TB resistant to at least isoniazid and rifampicin, plus any fluoroquinolone and at least one of three injectable second-line drugs. XDR-TB is considered a significant public health threat in Thailand because of the rising of reported XDR-TB cases, the more financial burden for both patient and provider sites, and more adverse events in XDR-TB treatment. This dissertation examines the biopsychosocial factors, such as a) whether non-adherence to the MDR-TB treatment regimen is a leading cause of development to XDR-TB, b) which NAT2 diplotype or HLA class 2 alleles associated with XDR-TB cases, c) the pattern of initial culture conversion in XDR-TB group while on MDR-TB treatment compare to MDR-TB group.

Methods: We conducted a matched case-control study and recruited all secondary XDR-TB cases

that were reported to the Bureau of Tuberculosis from October 2014 to June 2019. A total of 45 secondary XDR-TB cases were enrolled at 32 original hospital sites, across 29 provinces in Thailand. MDR-TB controls were randomly selected from the same hospital, where XDR-TB cases were treated. The proportion of cases to controls was one XDR-TB to two MDR-TB matched by geographical region. In the first two studies (Chapter 2-3), nine of 45 secondary XDR-TB cases died before we started. Then, only 36 XDR-TB cases and 75 MDR-TB controls were available for interviews and genetic tests. The conditional logistic regression was used to assess the magnitude of the non-adherence effect. Fisher exact test and conditional logistic regression were also used to explore the association between NAT2 diplotype, HLA class 2 alleles, and XDR-TB cases. In the third study (Chapter 4), we could not find the record of sputum culture in seven of 45 secondary XDR-TB cases. We used Cox proportional hazard regression for the difference in time to initial culture conversion in 38 XDR-TB cases and 76 MDR-TB controls. We also used the sensitivity and specificity of initial culture conversion at the beginning of MDR-TB treatment to 15-month in the prediction of the XDR-TB susceptibility and MDR-TB treatment outcome.

Results: We found that patients with non-adherence while on MDR-TB treatment were ten times more likely to develop XDR-TB (AOR 10.08, 95%CL 1.36, 74.87) while adjusted for type of Directly Observed Treatment, age, gender, socioeconomic status, and harmful alcohol use which was less than the magnitude from improper regimen (AOR = 26.89, 95%CL 7.39, 97.81) while adjusted for culture sending and region. For the genetic study, *NAT2*4/5B* (OR 3.9, 95%CI 1.2 13.0) and the heterozygosity of *HLA-DRB1 16:02* were about four times associated with XDR-TB cases (OR 3.7, 95%CI 1.2, 11.9). None of the associations was found in HLA DQB1. For the third study, the improper regimen for MDR-TB treatment was found to significantly reduce the chance of initial sputum culture conversion (aHR 0.24, 95%CL 0.15, 0.38) adjusted for wrong drug administration and dosage, and follow-up sputum culture. Furthermore, positive culture at the end of four-month of treatment was a good predictor for failure outcome (sensitivity 67.7%, specificity 93.8%) and the development of XDR-TB (sensitivity 63.2%, specificity 94.7%).

Conclusion: The main factor that causes XDR-TB was not non-adherence; the more significant factor was improper regimens for MDR-TB treatment, which mainly came from the provider site. From the aspect of host genetic, the result of this study was found significant in NAT2 *4/5B and heterozygosity of *HLA-DRB1 16:02*. There was no clinically significant for NAT2. However, *HLA-DRB1 16:02* is associated with acquired immunodeficiency that can make the host more likely to develop XDR-TB. For the follow-up of sputum culture conversion, the improper MDR-TB treatment regimen also played a significant role in reducing the chance of sputum culture conversion. Also, the lack of sputum culture conversion at month four would give us the best prediction for the development of XDR-TB. Overall, the finding from these three studies suggested the probable risk and protective factors of XDR-TB. From a policy perspective, further work is needed to examine the impact of the new DR-TB guidelines on the actual MDR-TB treatment regimen and assess the effectiveness of the treatment audit system when it starts.

The dissertation of Kaewalee Soontornmon is approved.

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LIST OF ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
AM	Amikacin
BDQ	Bedaquiline
BID	Twice a day
BMI	Body mass index
BTB	Bureau of Tuberculosis
CFZ	Clofazimine
CM	Capreomycin
CS	Cycloserine
CXR	Chest x-ray
DLM	Delamanid
DM	Diabetes mellitus
DOTS	Directly Observed Treatment, Short course
DST	Drug-susceptibility testing
EMB	Ethambutol
ETO	Ethionamide
FQs	Fluoroquinolones
HIV	Human immunodeficiency virus
INH	Isoniazid
IM	Intramuscular
IV	Intravenous
KM	Kanamycin
LFT	Liver function test
LFX	Levofloxacin
LMICs	Low-to-middle income countries
LPA	Line Probe Assay
LTBI	Latent tuberculosis infection
LZD	Linezolid

MDR	Multidrug-resistant
MFX	Moxifloxacin
NTM	Nontuberculous mycobacterium
OFX	Ofloxacin
PAS	Para-aminosalicylate
PZA	Pyrazinamide
RIF	Rifampin
SCC	Sputum Culture Conversion
SLDs	Second-Line Drugs
SM	Streptomycin
TB	Tuberculosis

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POSTER PRESENTATION

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Chapter 1. Introduction and background

1.1 Introduction

Tuberculosis (TB) is a communicable disease that is transmitted to other people by cough aerosol spread and caused by *Mycobacterium tuberculosis* complex. It is usually found in the lungs (≈85%), but extrapulmonary sites can be involved. Although TB emerged 70,000 years ago, we have not yet eliminated this disease because of several problems. One of them is the problem of drug resistance, where TB bacteria causing disease will not respond to at least one of the main anti-TB drugs (1). The degree of drug resistance defines whether TB is Multidrug-Resistant or Extensively Drug-Resistant.

Multidrug-resistant TB (MDR TB) is caused by an organism that is resistant to both isoniazid (INH) and rifampin (RIF), the two most potent TB drugs. These drugs are used to treat all persons with drug-susceptible TB. Extensively Drug-Resistant TB (XDR-TB), which was first defined in 2006, is a type of MDR TB that is resistant to INH and RIF, plus any fluoroquinolone (FQs) and at least one of three injectable second-line drugs (i.e., AM, KM, or CM) (2, 3).

TB is easily transmitted via droplet nuclei containing *M. tuberculosis*. If others inhale air containing these droplet nuclei, they may become infected. However, not everyone infected with TB bacteria becomes sick. As a result, two TB-related conditions exist, classified as latent TB infection and TB disease (4).

Given these two related conditions, latent tuberculosis infection (LTBI) is a state where there are detectable persistent immune responses to stimulation by *M. Tuberculosis* antigens with no signs

and symptoms of clinically active TB (5). The only sign of TB infection is a positive reaction to the tuberculin skin test or TB blood test (interferon-gamma release assay). Persons with latent TB infection are not infectious and cannot spread TB infection to others. Contrary to Latent TB infection, people with active TB disease have clinical symptoms or findings (i, e. fever, cough, night sweats, or unexplained weight loss) and are considered infectious to others if the site of disease is at pulmonary and larynx (6, 7).

Global situation of TB, DR-TB

The burden of drug-resistant TB is a massive concern at the global, regional, and country-levels. In 2018, half a million new cases of RIF-resistant TB were estimated, and 78% of them were diagnosed as multidrug-resistant TB. The three countries that contributed a large share of global burden were India, China, and the Russian Federation for 27%, 14%, and 9%, respectively. Moreover, the estimates of MDR/RR-TB among new TB cases was 3.4%, and among previously treated cases was 18%. The global treatment outcome data showed success rates of 56% for MDR-TB patients in the 2016 cohort, and this has increased in recent years from 50% in the 2012 cohort (the success rate at 50, 52, 54, 55, 56% from 2012 to 2016 cohort)(8).

Despite increased success in MDR-TB treatment, outcomes still demonstrate 8% drug failure, with15% of individuals dying from TB, and15% being lost to follow up (8). By the end of 2018, the average proportion of MDR-TB cases with XDR-TB was 6.2% (95% CI: 4.4–8.2%) (8), which is lower than the 9.5% from 2015 (9). This report represented data from continuous surveillance programs or surveys identifying the proportion of MDR-TB cases that included XDR-TB from 128 countries and five territories over the past 15 years.

Current situation of TB, DR-TB in Thailand

Currently, people are still struggling with TB disease, especially in Thailand, which is in the Southeast Asia region and has about 69 million inhabitants. TB is a serious health problem in Thailand, with the country being included among 30 nations that are part of the three high-burden country list (TB, TB/HIV, and MDR-TB), defined by WHO from 2016 to 2020 (8). Between 2015 and 2018, the estimated trend of MDR/RR TB among new cases in Thailand was 2.2-2.3%, and among previously treated patients was 24%. Treatment outcomes showed a success rate of 60-61% for MDR-TB patients in the 2015 and 2016 Thai cohorts. Based on the WHO report, the number of laboratory-confirmed XDR-TB cases in Thailand rose from 5 in 2015 to 29 cases in 2018 (2, 8-10).

The national TB control program is coordinated by the Bureau of TB Control (BTB), which is under the Department of Disease Control (DDC) of the Ministry of Public Health. The Department of Disease Control is further broken down into thirteen regional Offices of Disease Prevention and Control, which in turn coordinate a network of Provincial Health Offices. (PHO). Public health care services are organized in three tiers: provincial hospitals (400-2,000 beds), district hospitals (30-120 beds), and health centers (no beds or resident doctors). Also, tertiary public hospitals attached to Universities treat TB and MDR-TB patients (e.g., Srinagarind University Hospital in Khon Kaen); these fall under the Department of Education. In parallel, TB care is also dispensed through a diversified network of private providers and facilities (6).

BTB is responsible for TB policy development, surveillance, monitoring and evaluation, training and supervision down to the regional level, laboratory reference functions, and research. A National Expert Committee on DR-TB (NEC) was appointed in 2013 under the DDC with a

secretariat in the BTB to tackle the increasing trend of Drug-Resistant TB. This technical committee meets every 2-3 months; it is composed of 10 members with expertise covering clinical, public health, and laboratory aspects (6).

The NEC has three roles: 1) policy-making and development of guidelines, 2) advisory on MDR-TB issues for doctors, and 3) review of all XDR-TB patients and other cases for whom medication for treating XDR-TB is required. In order to control the usage of the novel anti-TB drugs, all drugs for treating XDR-TB were only distributed from BTB after approval from NEC. Starting in April 2014, the first case of XDR-TB was sent to BTB to request medications for XDR-TB treatment. However, capreomycin (CM), linezolid (LZD), clofazimine (CFZ), and moxifloxacin (MFX) were available in October 2015. Later, bedaquilline (BDQ) became available in March 2016.

In February 2018, XDR-TB was announced as a dangerous communicable disease under the communicable disease act BE 2015. According to this communicable disease act, both suspected and confirmed XDR-TB cases have to be reported to the Department of Disease Control via BTB. Since 2018, BTB has distributed second-line Line Probe Assay for diagnosing XDR-TB to 13 regional laboratories. However, second-line phenotypic DST was available only at the National TB Reference Laboratory (NTRL).

1.2 Gaps in the literature.

Given more toxic side-effects from XDR TB treating regimen, high cost of XDR-TB treatment (11), and the low treatment success rate in XDR-TB (39%in global TB report) (8). It is critical to know the potential risk factors for MDR-TB patients to develop dangerous drug-resistance (XDR-TB) in their course of treatment. The risk factors for the development of XDR-TB have been studied in many clinical and programmatic studies. In addition to the biomedical aspect, many studies also

explored the role of psychological support on treatment outcomes in low-to-middle income countries. However, few studies have examined the biomedical and psychosocial aspects together. In order to address the biopsychosocial factors associated with the development of XDR-TB, we conducted a case-control study that may help us improve the MDR-TB treatment system and lead to a reduction in the number of patients with XDR-TB.

1.3 Overall objective and specific aims

The purpose of this dissertation is to assess the risk factors of XDR-TB development by examining whether non-adherence is the leading cause of XDR-TB and explore the association between the genetic profile of NAT2 and HLA class 2 (especially DRB1, DQB1) and XDR-TB. We also observe the difference in the time to sputum culture conversion and the pattern of sputum culture conversion in both groups treated with MDR-TB treatment.

The first aim (Chapter 2) is to examine whether non-adherence is the primary cause of XDR-TB development. At the same time, we take other factors from the clinical aspect (proper regimen), programmatic management, and psychosocial support into account. We hypothesize that non-adherence might not be the major risk factor of XDR-TB development, and other causes should be explored to provide optimum care to TB patients (patient-centered care).

The second aim (Chapter 3) is to examine whether NAT2 diplotype or acetylation type is associated with XDR-TB. Moreover, we test whether the HLA class 2 in the form of alleles and carriers are associated with XDR-TB susceptibility. We hypothesize that either rapid or slow/ultraslow acetylator type might be found more in cases with XDR-TB than MDR-TB. The specific polymorphism in HLA class 2 might potentially be associated with XDR-TB.

The third aim (Chapter 4) is to observe the difference in the pattern of sputum culture conversion and reversion in both groups while treated with the MDR-TB episode. We also explore the cut-off point of sputum culture conversion to predict the chance to develop XDR-TB accurately. We hypothesize that the delay of sputum culture conversion might affect by improper regimen used and too short duration of intensive treatment.

Chapter 2. Is non-adherence is the primary cause of XDR-TB development

2.1 Abstract

Introduction: Drug resistance could come from the inappropriate use of TB medicines and poor-quality drugs. The clinical management for patients with XDR-TB is challenging because of the expensive cost of treatment, the highly toxic adverse reactions, and the poor treatment outcomes in XDR-TB. To reduce the burden of XDR-TB cases, there have been several efforts to study the potential factors that drive MDR-TB patients to develop XDR-TB. Nonetheless, the comprehensive study of biopsychosocial factors has not been taken into consideration at the same time. In this study, we provide the first evaluation of biomedical, programmatic, and psychological factors impacting XDR-TB development.

Methods: We conducted a matched case-control study and recruited all secondary XDR-TB cases that were reported to the Bureau of Tuberculosis from October 2014 to June 2019. A total of 45 secondary XDR-TB cases were enrolled at 32 original hospital sites, across 29 provinces in Thailand. MDR-TB controls were randomly selected from the same hospital, where XDR-TB cases were treated. The proportion of cases to controls was one XDR-TB to two MDR-TB. In this study, nine of 45 secondary XDR-TB cases died before we started. Then, only 36 XDR-TB cases and 75 MDR-TB controls were available for interviews. The conditional logistic regression was used to assess the magnitude of the non-adherence effect.

Results: We found that patients with non-adherence while on MDR-TB treatment were ten times more likely to develop XDR-TB (AOR 10.08, 95%CL 1.36, 74.87) while adjusted for type of Directly Observed Treatment, age, gender, socioeconomic status, and harmful alcohol use which was less than the magnitude from improper regimen (AOR = 26.89, 95%CL 7.39, 97.81) while adjusted for culture sending and region. In addition, family emotional support showed a significant protective effect for XDR-TB (COR= 0.30, 95%CL 0.09, 0.95).

Conclusion: the significant factors associated with XDR-TB in a high-burden and middle-income country like Thailand were improper regimens for MDR-TB treatment on the provider side, followed by non-adherence from the patient side. From a policy perspective to minimize the improper regimen in MDR-TB treatment, the effective consultation panel at the level of regional expert committees that collaborate with the national expert committee should be strengthened to provide optimal DR-TB treatment from the beginning of treatment. In addition, a treatment audit system should be in place and regularly conducted by the national expert committee.

2.2 Introduction

The emerging incidence of drug resistance could come from the inappropriate use or inappropriate prescription of TB medicines, poor quality drugs, or premature discontinuation of TB treatment (4). Anti-TB medications (Streptomycin) have been used since 1944 (12). Later, thioacetazone and para-aminosalicylic acid (PAS) started being used in 1948, with INH becoming available in 1952. During that time, the key to the prevention of further drug resistance was combination chemotherapy, which required 18 months of treatment. Fortunately, following the discovery of RIF in 1957, the most powerfully sterilizing anti-TB drug, shorter and more effective INH- and RIF-containing regimens were developed. These became known as short-course chemotherapy for drug-susceptible TB (13).

Despite the promise of short-course chemotherapy, this regimen is not effective for the treatment of MDR-TB patients. MDR-TB treatment is painful because the second-line TB drugs are weak and toxic (14). All regimens should include a later generation FQ, an injectable agent (KM, AM), and other oral second-line drugs (ETO, CS, PAS). Pyrazinamide (PZA) is routinely included in MDR regimens in resource-limited settings because DST to PZA is not widely available. Any drugs used in a regimen that did not cure patients with TB in the first place are considered ineffective even if recent DST showed that the patient's strain was still susceptible (13).

The first guideline for MDR-TB was developed in 2005 (15) consisted of an intensive phase with KM, OFX, PAS, EMB or ETO, and PZA of only three months, then the continuation phase, including all drugs above except KM. Later, the Bureau of Tuberculosis developed three more guidelines in 2013, 2015, and 2018 respectively, with further details on MDR-TB treatment (16-18). These three guidelines recommended the use of KM, LFX, ETO, CS, with/without PAS and

PZA. For the course of treatment, an intensive phase of 6-8 months and at least four months past culture conversion and a total treatment duration of 20 months and a minimum total length of treatment of 18 months after culture conversion were suggested for most patients (16-18).

In addition, guidelines for XDR-TB treatment in Thailand (17) need at least four effective drugs that never used before or had susceptible results from the DST report. The recommended XDR-TB regimen, which consisted of MFX (if reported susceptible), LZD, CFZ, CM, or other new drugs (BDQ, DLM), has to be maintained at least 20 months. The severe side effects were reported more in this XDR regimen. The serious adverse event associated with linezolid is optic neuritis, peripheral neuritis, and myelosuppression. The most common adverse event associated with BDQ is a QTc interval increase on electrocardiogram, and even increase in QT prolongation with BDQ and CFZ combination (17, 19).

As mentioned above, the clinical management of patients with XDR-TB becomes even more challenging. The reasons include a lack of potent anti-TB drugs, expensive cost of treatment, highly toxic adverse reactions (11), and poor treatment outcomes in XDR-TB (8). There were several efforts to study the potential factors that drive MDR-TB patients to develop XDR-TB in order to reduce the burden of XDR-TB cases. Many factors for the development of XDR-TB have been evaluated in clinical and programmatic studies.

First, one study from France from 1998 to 2013 showed that patients with MDR-TB who had foreign birth, previous anti-TB treatment, smear positivity, and EMB resistance were more likely to develop XDR-TB, and had more frequent need of rapid molecular testing for SLDs (20). Second, another study from the Russian Federation between 2000 and 2004 revealed that six percent of MDR-TB patients developed XDR-TB. Risk factors included the presence of bilateral cavitary

lesions, prior exposure to second-line injectable antibiotics, and incremental risk for each additional month in which a patient failed to take at least 80% of their prescribed drugs (21). Third, one additional study from Russia from 2005 to 2008 showed that patients who received less than three effective drugs for TB treatment were more likely to acquire XDR-TB (22).

In Thailand, we found two studies that describe the characteristics and treatment outcomes in MDR -TB groups of patients, demonstrating highly unfavorable results such as death or loss to follow up (23, 24). Nevertheless, no risk factor of XDR-TB was identified, such as improper regimen or non-adherence.

Patients who always miss doses may lead to the emergence of MDR/XDR-TB, and prolong the duration of infectiousness, thus facilitating TB transmission to the public (25). Non-adherence may also affect treatment outcomes. These characteristics were mainly patient-related factors such as alcoholism, illegal drug use, adverse drug reaction, history of negligence, lack of social support, and lower socioeconomic status (26-31) and related programmatic factors such as miscommunication between patients and health care providers, and hard to access health care services (32-34). Specifically, in MDR-TB treatment default (35), Holtz and colleagues found that loss to follow-up was most strongly predicted by substance abuse, dissatisfactory attitudes about health care workers, and indicators associated with low socioeconomic status (35).

In addition to the biomedical aspects, many studies also explored various psychological support factors on treatment outcomes in low-to-middle income countries. For example, psychotherapy during treatment was proven to improve adherence and cure outcomes in India (36). Group based psychological support improved treatment outcomes in MDR-TB groups (37). Many studies also

revealed that a psychosocial approach in patient-centered TB care platforms might result in better treatment outcomes (38).

Previous studies that reported risk factors of XDR-TB in the past always report either solely biomedical, programmatic, or psychological aspects. However, the comprehensive study of biopsychosocial factors has not been taken into consideration at the same time. In this study, we provide the first evaluation of biomedical, programmatic, and psychological factors impacting non-adherence. Furthermore, we assess whether non-adherence is the primary risk factor for the development of XDR-TB in the context of Thailand.

2.3 Methods

Study design and population

We conducted a case-control study and recruited all secondary XDR-TB cases that were reported to the Bureau of Tuberculosis (BTB) from October 2014 to June 2019. The total reported XDR cases in Thailand were 56 cases (Primary: 11 cases, Secondary: 45 cases). Specific for secondary XDR-TB, nine of 45 cases died before we started. A total of 36 secondary XDR-TB cases were enrolled at 32 original hospital sites, across 29 provinces in Thailand. Next, we asked health staff from the TB clinic to randomly select MDR-TB controls from the same hospital where XDR-TB cases were treated, from 2015 to 2018. The proportion of cases to controls was one XDR-TB to two MDR-TB.

Case definition: Secondary XDR-TB cases (36 cases) were all TB patients who had a history of anti-TB treatment for more than one month with culture- or molecular-proven XDR-TB registered in any hospital in Thailand. Specimens from these patients were sent to The National TB

Reference Laboratory (NTRL) for second-line drug susceptibility testing. Control definition: MDR-TB in the control group consisted of patients diagnosed with MDR-TB who did not later develop XDR-TB in the fiscal year of 2015-2019 and matched by province or public health region as the case. With double the number of controls (75 controls) being enrolled.

Inclusion criteria: XDR-TB patients registered for the treatment of XDR-TB (cases) patients with MDR-TB (controls), who provided written informed consent. *Exclusion criteria*: age less than 18 years old, prisoner, non-Thai, and refusal to participate.

With the total sample size of 111 (36 XDR-TB cases and 75 MDR-TB controls), this study has about 80% power to detect a difference in non-adherence while on MDR-TB treatment between secondary XDR-TB cases and MDR-TB controls. The sample size of this study was calculated based on using a 1:2 matched design by geographical area, with a 95% confidence interval (two-sided), and alpha at 0.05. By the end of 2016, The percentage of estimated XDR-TB cases in an MDR-TB cohort study in the Philippines who was lost to follow up (LTFU) was 42.5% (39), whereas the proportion of MDR-TB cases with XDR-TB was 6.2% from global TB report (at the end of 2016) (2). Then, the OR for the development of XDR-TB in both cohorts was 6.85. The estimated number of XDR cases needed to achieve 80% power in the present study is 36. We performed sample size calculations using STATA 15 (StataCorp, College Station, TX, USA).

Data collection and tools

There were two standardized forms used in this study. The first was the *case record form* that was developed from the XDR-TB request forms, which are included in the guidelines for prevention and control of Extensively Drug-Resistant TB in Thailand under the communicable disease act of 2015 (11). This form is used to collect socio-demographic, clinical, and laboratory data from the

medical charts of patients. The second form was *a semi-structured questionnaire* that asked about patient-reported adherence, behavior, harmful alcohol use, tobacco use, socioeconomic characteristics, and psychosocial support. **We collected all the data while both groups of patients received MDR-TB regimens.**

First, eligible TB patients were contacted by the TB coordinators in the selected hospitals either in person or through a telephone call. The participants were informed of the study and had a chance to decide whether they would participate or not. For the patients who were willing to participate, we abstracted their data from clinical reporting forms that were completed by TB health staff on the appointment date. Moreover, we completed the case record forms by retrospectively reviewing electronic health records, medical charts, and laboratory databases.

After reviewing the health record, patient interviews were conducted based on the appointment date after patients had visited the TB clinic for their treatment. TB patients provided signed informed consent before the audio-recorded interview started. Trained research assistants conducted the semi-structured interview in Thai, each interview lasting for 30–40 minutes. The semi-structured interview guides were developed after consulting with health researchers from the Society and Health Institute, Ministry of Public Health.

Questionnaires were then pre-tested and fine-tuned by the BTB staff and research assistants. The questionnaire covered 1) socio-demographic data, 2) information about adherence to treatment, 3) their DOT watchers, 4) any difficulty they experienced while on MDR-TB treatment, 5) their attitude about health care, and 6) social factors affected adherence such as harmful alcohol use, smoking, and drug abuse. For XDR-TB cases, interviewers would probe participants to answer

specific items at the time they were treated with the MDR-TB regimen. The questionnaire was developed and pilot-tested by ten MDR-TB patients before the actual data collection.

Operational definition and classification

Non-adherence: Missing anti-TB medication more than 10 % of the total intended course. (40, 41)

Default or loss to follow-up: Patients not taking anti-TB drugs/not visiting TB clinic for two months or more, consecutively after starting treatment. (16, 18)

Improper regimen: Insufficient number of medications or shorten the duration of treatment than it was recommended in MDR-TB treatment guideline (2013). An insufficient number of drugs defined as the intensive phase consists of less than four effective second-line anti-TB drugs or if the continuation phase consists of less than three effective drugs.

Adequate duration: A 6-8-month intensive phase with at least four months past culture conversion was recommended as the treatment duration for most patients. 20-month total treatment duration with at least 18 months after culture conversion was recommended as the total duration of treatment for most patients (17, 42).

Improper administration and dosage: *Anti-TB drugs* are not prescribed in the normal dosage range or proper interval to achieve the peak concentration with a minimal side effect.

The anti-TB dosage is adjusted based on the weight of TB patients. Typically, dosing tables with a few weight bands will be used. When adults gain/ lose weight and move into different weight bands, drug doses will be adjusted accordingly (16, 17, 43, 44). *For the administration*, most anti-

TB drugs will be taken once daily dose in order to receive peak concentration; however, ETO and CS are typically given in two divided doses to reduce side effects. Especially in the CS peak concentration has to be kept under 35 mcg/ml in order to prevent most serious CNS side effects usually occur at peak concentrations (C_{max}) >35 mcg/ml (45).

After a review of dosing guidelines for second-line anti-TB drugs from the Thai National TB and the WHO guidelines (16, 17, 43, 44), the normal dosage will be determined for the study. The normal dose for KM is 12-15 mg/kg/day, LFX 10-15 mg/kg once daily, ETO/PTO 15-20 mg/kg/day, CS 10-15 mg/kg/day, PAS 150-200 mg/kg/day. The 750 mg once-daily dose of CS, which gives the C_{max} value at 50 mcg/ml, will be defined as the wrong administration (46).

Nutritional status: According to the regional office for the western pacific (WPRO) standard which is the recommended standard for the Thai population (47), underweight is defined as a BMI < 18.5kg/m^2, normal weight refers to a BMI between 18.5 and 22.9kg/m^2, and overweight refers to a BMI between 23.0 and 24.9kg/m^2, and obese refers to a BMI ≥ 25kg/m^2.

Diabetic control: According to the Thai clinical practice guideline for Diabetic 2017 (48), our study defined well control DM as an HbA1C ≤ 7.0%. **Poor glycemic control** was defined as an HbA1C > 7.0% or a history of hyper or hypoglycemia (48, 49).

Harmful alcohol use: A score of 8 or more is defined as harmful or hazardous drinking based on the Alcohol Use Disorders Identification Test (AUDIT). AUDIT is a 10-item screening tool developed by the World Health Organization (WHO)(50) to assess alcohol consumption, drinking behaviors, and alcohol-related problems.

Data analysis

Data from the case record form and the questionnaire were entered into the data entry form. Data entry used a double data entry method in Excel (Microsoft Corp., Seattle, WA), then imported and analyzed in SAS version 9.4 (SAS Institute, Inc., Cary, NC). Medians with interquartile ranges (IQRs) were calculated for continuous variables, and frequency distributions were tabulated for categorical variables. Groups were compared using the two-sample Wilcoxon-Mann-Whitney test for continuous data and Chi-square/Fisher's exact test for categorical data. Before assessing the association by logistic regression, we conducted stratified bootstrap resampling for 250 times to find a 2.5^{th} and 97.5^{th} percentiles confidence interval for each variable in univariate analysis. Univariate logistic regression was used to identify factors associated with XDR-TB. We chose the significant variables which P-value <0.20 or related from prior knowledge into a multivariable model (20, 21, 51). The conditional logistic regression was used to determine the effect estimate matched by region of participants.

Ethics statement

The Institutional Review Board of the University of California Los Angeles (UCLA IRB#19-000158) and the Ethics Committee for Research in Human Subjects Department of Diseases Control (FWA #00013622) approved this study in 2019. This study was also approved by ten local Institutional reviewed boards from selected hospitals. In addition, 22 hospitals approved the informed consent forms before we conducted the study in their hospitals.

2.4 Results

We researched in order to assess whether non-adherence to the treatment regimen is a significant risk factor in the development of XDR-TB.

2.4.1 Demographic data

Overall, 36 case participants were diagnosed with XDR-TB, and 75 control participants were diagnosed with MDR-TB, for a total of 111 subjects, aged between 18-82 years. The baseline demographic by drug-resistance patterns was described in table 2-1. The median age of MDR-TB controls was 47 years (interquartile range IQR [34-59]), while the median age of XDR-TB cases was 40 years (IQR [28-51]). We found that 75% of XDR-TB cases were male and that 67% of MDR-TB cases were also male. The proportion of XDR-TB cases who lived in Bangkok and the big cities was 25%, which was higher than MDR TB controls (17.3%). Also, we found that the proportion of patients of middle and high socioeconomic status while on MDR-TB treatment was 47.2% for those who later developed XDR-TB as opposed to 32% in MDR-TB patients. No factor in table 2-1 was significantly different except the median age during the MDR-TB episode, which was lower in cases of MDR-TB that later developed into XDR-TB (P-value=0.0083).

2.4.2 Biological and clinical characteristics associated with the development of XDR-TB

In terms of HIV co-infection, six out of 111 patients (5.4%) were co-infected with HIV (one case in the XDR-TB group). We observed that 20 of 36 patients (55.6%) in the XDR -TB group were underweight, which was higher than 33 of 75 (44%) in the MDR-TB group. One-third of the cases were diagnosed with diabetes mellitus (DM), and eight of ten DM cases (80%) in the XDR-TB group were poorly controlled, while only 10 of 23 patients with DM in the MDR-TB group were poorly controlled (43.5%).

Cavities were observed in the CXR while on MDR-TB treatment more frequently among patients in the XDR-TB group than those who remained with an MDR-TB diagnosis (61% vs. 44%). Smear positivity, while starting MDR-TB treatment, was found more frequently in patients who developed XDR-TB than among patients in the MDR-TB group (75% vs. 54.7%). When we compare the

episode of treatment between the two groups, we found that a higher proportion of people with XDR-TB experienced more than one episode of MDR-TB treatment (30.6% vs. 2.7%). Only those patients with XDR-TB experienced three and four episodes of MDR-TB treatment (four and two cases, respectively).

We collected information about adherence from the interview and chart-reviews. After we assessed a correlation between both methods, we found a weak correlation between chart-review and interview ($|r| = 0.19$) (figure2-1). Therefore, we created a new variable that captured how frequently patients missed the dose by choosing the maximum number of missed doses from the chart-review or interviews. The patients who missed doses of more than 10 % of the total MDR-TB intended course (Non-adherence) were nine times more likely to develop XDR-TB later. (COR 9.27, 95%CL 2.81, 30.61).

The non-adherence occurred 40% in the first six months and 35% in the seventh to the fifteenth month of treatment. The pattern of missing doses was mostly consecutive (70%). Only five percent of participants missed their PAS drug for the regimen due to a stockpile problem. In addition, the patients who received an improper regimen while on MDR-TB treatment more frequently developed XDR-TB later (COR=31.61, 95% CL 8.76, 114.01).

When MDR-TB treatment was started, we also found that being smear-positive at the beginning of the MDR-TB treatment phase (COR= 4.55, 95% CL 1.25, 16.57) was more likely to be associated with later development of XDR-TB. While on MDR-TB treatment, we found significantly fewer side effects, such as hearing loss and vestibular disturbances (COR=0.37, 95% CL 0.14, 0.96), and arthralgias (COR=0.38, 95% CL 0.15, 0.93) in the MDR-TB cases, who subsequently developed XDR-TB.

2.4.3 The association of psychosocial factors with XDR-TB development

When comparing the 36 XDR-TB patients with the 75 MDR-TB patients, a higher rate of continued work while on MDR-TB treatment was observed among XDR-TB cases as compared to MDR-TB (69.4% vs. 41.3%) and was three times more likely to develop XDR-TB (COR = 3.52, 95%CL 1.45, 8.58). Furthermore, we combined work while on MDR-TB treatment with no adverse events (hearing loss/vestibular disturbance, hypothyroid, and arthralgia) together as one variable. Patients who were working and not exposing with the above three side effects while on MDR-TB treatment had six times higher chance of non-adherence (COR = 6.43, 95% CL 2.12, 19.48) and developing XDR-TB later (COR = 5.98, 95% CL 2.23, 16.07). In addition, six patients with the above characters and who continued drinking alcohol all developed XDR-TB.

Continued alcohol drinking while on MDR-TB treatment was associated with patients with non-adherence (COR = 5.83, 95% CL 1.65, 20.68) and XDR-TB (COR = 5.17, 95% CL 1.54, 17.72). However, a history of smoking more than 30 packs of cigarettes per year (COR = 5.83, 95 CL 1.58, 21.57) was found to be significantly associated only with developing to XDR-TB.

Family emotional support seemed to be a protective factor against the development of XDR-TB (COR = 0.30, 95% CL 0.09, 0.95). Financial and educational support did not seem significantly different between the two groups. More than 95% of patients in both groups were satisfied with their healthcare providers, and 90% of them thought that health care access was convenient. In summary, we found no differences in health care attitudes and behavioral questions among MDR and XDR-TB groups of patients.

For directly observed treatment (DOT), participants with DOT by solely healthcare workers were five times more likely to develop XDR-TB (COR = 5.50, 95% CL1.70, 17.76). However, it was not associated with non-adherence and a difficult commute while on the MDR-TB treatment. On the contrary, DOT by a family member played a protective role in the development of XDR-TB (COR = 0.35, 95%CL 0.13, 0.92). We did not see the difference among groups who did or did not receive DOT by village health volunteers.

After considering the match for the region of the hospital in the logistic regression model, the odds ratio and 95% confidence intervals for each variable were quite similar to the effect from unconditional logistic regression (table 2-4).

2.4.4 Effect of adherence and improper regimen on XDR-TB Development

After multiple logistic regression analysis (table 2-5), the effect of non-adherence (AOR = 8.94, 95%CL 1.29, 62.21) remained significantly associated with the development of XDR-TB when adjusted for DOT by solely healthcare workers, age, gender, SES, and harmful alcohol use (table 2-5, model 3). In addition, the odds ratio estimates from conditional multiple logistic regression (table 2-5, model 4) reported the effect of non-adherence (AOR = 10.08, 95%CL 1.36, 74.87).

For the improper regimen, we controlled the factor of culture sending and region in the final model. The odds ratio estimates from conditional multiple logistic regression reported the effect of the improper regimen was about 27 times more likely to develop XDR-TB (AOR = 26.89, 95%CL 7.39, 97.81).

2.5 Discussion

We compared the characteristics of patients who were treated with MDR-TB regimens and who later developed XDR-TB (previous MDR-TB patients who developed XDR-TB in 2015-2019) and patients who remained or succeeded in the MDR-TB group. The results of our study highlighted some factors that could lead to the development of XDR-TB. We identified several factors for the development of XDR-TB while on MDR-TB therapy by using model three to close biasing paths in the directed acyclic graph (figure 2-2). In models one to three, we added more variables to achieve all variables in model three. The variables included in model three consisted of improper regimen, DOT by solely healthcare workers, SES, age, gender, and harmful alcohol use. However, the point estimate was quite similar in models two and three, so we assumed that the biasing path from not controlling harmful alcohol use might create a small degree of bias. While comparing models three and four, the result also quite similar.

First, an inadequate number of active drugs in the regimen and proper duration (improper regimen) was a significant risk factor for XDR-TB development. A study from Russia also had similar findings in that acquired drug-resistance and XDR-TB were seen more frequently in patients who received less than three effective drugs (P-value =0.03)(22). In our study, more than 90% of XDR cases received less than four effective drugs in the intensive phase of treatment and less than three effective drugs in the continuation phase while on MDR-TB treatment. Nonetheless, 11 cases out of 36 (30%) who later developed XDR-TB disease started their MDR-TB regimen before 2013. Although standard protocol for MDR-TB treatment has been in place in Thailand since 2005, revised to the standard regimen in 2013, and last updated in 2018, many physicians might not follow the standard protocol. The reason for not following standard protocol

recommendations could be explained by lack of experience and updated training of TB staff, which was similar to previous studies (52-54), and an inadequate stockpile of drugs.

Second, non-adherence to MDR-TB treatment was the second risk factor for developing XDR-TB. Overall, a high adherence rate of 85% was shown in both groups, which is likely due to the implementation of DOT in many parts of Thailand, especially in the southern area. Participants who missed more than 10% of the total intended course were about ten times more likely to develop XDR-TB than the group who adhered to treatment. In the Russian study on XDR-TB development conducted between 2000 to 2004, the presence of incremental months of failed adherence where more than 20% of doses were missed demonstrated an additional 20% hazard of XDR-TB development (21).

From the univariate analysis, patients who received only healthcare delivery of DOT were more likely to develop XDR-TB. However, this result might be affected by several factors—first, an ineffective system of directly observed therapy contributing to poor treatment outcomes (52). Second, healthcare staffs were more likely to choose patients who were at higher risk of developing XDR-TB for healthcare delivered DOT. In addition, DOT provided by overworked healthcare personnel might not be the best option to promote patient adherence.

In 2014, Ershova et al. reported that patients receiving partial DOT during treatment or not receiving DOT had higher proportions of poor outcomes than patients who received DOT during the entire course of treatment (52). Two studies about healthcare delivery of DOT in Thailand reported that health care personnel might not be an excellent choice for DOT in some settings due to weak sustainability. The actual practice of DOT was different from the assignment at the

beginning of treatment and was not related to the treatment success in the southern part of Thailand in 2002 (55, 56).

However, another study from Thailand in 2008 showed the contrasting finding that the magnitude of benefit in treatment outcome was greater among DOT by healthcare personnel (57). Although the policy was to convince all TB clinics to use DOT by healthcare or VOT (Video observed treatment) method, 40% of participants in this study reported only self-administration of TB treatment pills on their own.

Smear positivity was related to XDR-TB development in our cohort (COR=3.03. 95%CL 1.03, 8.94). The study in France by Guglielmetti et al. also showed that smear positivity was associated with XDR-TB development. This association could be explained by the fact that patients in the XDR-TB group experienced previous MDR-TB treatment episodes and had a high bacillary load with a much higher likelihood of mutations (20).

In addition, family emotional support was associated with preventing XDR-TB development. Most studies in low and middle-income countries also support these results. XDR-TB patients need psychosocial support because some of them might experience some traumatic events such as discrimination from their family members, their colleagues, and the community. However, if they all were educated about TB disease, side effects of anti-TB medication, and how to support patients, the proper care and psychological support from family and community would increase the success of treatment outcomes. Family emotional support was the most important in terms of caring for daily patient routines and regular encouragement. (36-38, 58, 59)

We noticed that six patients who a) did not experience three significant adverse treatment effects, b) continued to work in order to maintain their socioeconomic status, and c) continued alcohol drinking were more likely to miss dose > 10% and develop XDR-TB later. These findings highlight the difficulty in managing some specific groups of patients. Healthcare providers should investigate alternative options for the delivery of optimal patient-center care in this particular group of patients. Communicating with all stakeholders is critical in order to provide patients with appropriate resources and tools to address stressors, including financial need and alcohol abuse.

Our study had three main limitations. First, there were only 36 secondary XDR-TB cases in this study. Therefore, it can create sparse data bias; however, we did use firth bias adjustment in the analysis to minimize this problem. Second, XDR-TB patients might have forgotten some past events while they were on MDR-TB treatment (recall bias), but the interviewer had the time frame when they were treated for the MDR episode and tried to probe them to remember that time. Third, we could not identify whether the XDR-TB strain in each patient came from acquired infection from previous MDR-TB treatment or was a newly transmitted XDR-TB strain from other patients (new-infection). Molecular typing will help us identify this issue.

In conclusion, the significant factors associated with XDR-TB in a high-burden and middle-income country like Thailand were improper regimens for MDR-TB treatment on the provider side, followed by non-adherence from the patient side. Recognizing the characteristics and factors that may cause patients to be more prone to XDR-TB development should trigger healthcare providers to promptly attempt to identify XDR-TB and to prescribe effective regimens while awaiting DST results. Healthcare providers may start comprehensive care by engaging stakeholders, delivering educational materials, and providing psychosocial support for MDR-TB infected individuals.

From a policy perspective to minimize the problem of improper regimen, the effective consultation panel at the level of regional expert committees that collaborate with the national expert committee should be strengthened to provide optimal DR-TB treatment from the beginning. In addition, a treatment audit system should be in place and regularly conducted by the national expert committee in order to assess the effectiveness of prescribed MDR-TB regimens. BTB may develop a set of practical knowledge tools on MDR-TB for patients, families, and communities.

2.6 Tables and figures

Table 2-1 Baseline demographic by drug-resistant pattern (N=111)

			Drug-resistant pattern			
	Overall		MDR-TB		XDR-TB	
	N	(%)	N	(%)	N	(%)
Age group (years)at diagnosis						
15-35	37	(33.3)	21	(28.0)	16	(44.4)
36-55	49	(44.1)	33	(44.0)	16	(44.4)
56-85	25	(22.5)	21	(28.0)	4	(11.1)
Age (median, IQR) at diagnosis	42 (32, 55)		47 (34, 59)		40 (28, 52)	
Gender						
Female	34	(30.6)	25	(33.3)	9	(25.0)
Male	77	(69.4)	50	(66.7)	27	(75.0)
BMI Category						
Underweight	53	(47.7)	33	(44.0)	20	(55.6)
Optimal	41	(36.9)	29	(38.7)	12	(33.3)
Overweight	10	(9.0)	7	(9.3)	3	(8.3)
Obese	7	(6.3)	6	(8.0)	1	(2.8)
Region						
Central	34	(30.6)	23	(30.7)	11	(30.6)
East	12	(10.8)	8	(10.7)	4	(11.1)
North	9	(8.1)	6	(8.0)	3	(8.3)
Northeastern	20	(18.0)	14	(18.7)	6	(16.7)

			Drug-resistant pattern			
	Overall		MDR-TB		XDR-TB	
	N	(%)	N	(%)	N	(%)
South	21	(18.9)	14	(18.7)	7	(19.4)
West	15	(13.5)	10	(13.3)	5	(13.9)
Address						
Bangkok and big city	22	(19.8)	13	(17.3)	9	(25.0)
up-country urban	34	(30.6)	23	(30.7)	11	(30.6)
up-country rural	55	(49.5)	39	(52.0)	16	(44.4)
Marital status						
Single	20	(18.0)	12	(16.0)	8	(22.2)
Married	61	(55.0)	40	(53.3)	21	(58.3)
Widowed	7	(6.3)	6	(8.0)	1	(2.8)
Divorced/Separate	23	(20.7)	17	(22.7)	6	(16.7)
Education level						
No standard education	4	(3.6)	3	(4.0)	1	(2.8)
Below High school	45	(40.5)	30	(40.0)	15	(41.7)
Junior High school	18	(16.2)	10	(13.3)	8	(22.2)
Senior High school/ Vocational certification	26	(23.4)	20	(26.7)	6	(16.7)
Diploma or higher	18	(16.2)	12	(16.0)	6	(16.7)
Occupation						
Agriculture/fishery	8	(7.2)	7	(9.3)	1	(2.8)
Business owner	19	(17.1)	12	(16.0)	7	(19.4)

			Drug-resistant pattern			
	Overall		MDR-TB		XDR-TB	
	N	(%)	N	(%)	N	(%)
Unskilled labor	32	(28.8)	23	(30.7)	9	(25.0)
Employee	39	(35.1)	24	(32.0)	15	(41.7)
Government/state enterprise officer	3	(2.7)	2	(2.7)	1	(2.8)
Housewife	4	(3.6)	2	(2.7)	2	(5.6)
Student	2	(1.8)	2	(2.7)	0	0
Retired	2	(1.8)	1	(1.3)	1	(2.8)
Priest	2	(1.8)	2	(2.7)	0	0
SES classes while on MDR tx						
Poor	70	(63.1)	51	(68.0)	19	(52.8)
Middle	18	(16.2)	9	(12.0)	9	(25.0)
Rich	23	(20.7)	15	(20.0)	8	(22.2)

Note: the percentage may not sum up to 100% because of missing value.

Table 2-2 Biological and clinical characteristic by drug-resistant pattern (N=111)

	Overall		Drug-resistant pattern			
			MDR-TB		XDR-TB	
	N	(%)	N	(%)	N	(%)
The proportion of non-adherence						
adherence	94	(84.7)	71	(94.7)	23	(63.9)
non-adherence (missed>10%)	17	(15.3)	4	(5.3)	13	(36.1)
Site of TB						
Pulmonary	105	(94.6)	70	(93.3)	35	(97.2)
Both pulmonary and extrapulmonary	6	(5.4)	5	(6.7)	1	(2.8)
Cavitary lesion	55	(49.5)	33	(44.0)	22	(61.1)
Smear positivity						
NA	16	(14.4)	11	(14.7)	5	(13.9)
Neg	29	(26.1)	23	(30.7)	6	(16.7)
Scanty-1+	26	(23.4)	16	(21.3)	10	(27.8)
2+-3+	40	(36.0)	25	(33.3)	15	(41.7)
The culture sent according to guideline	19	(17.1)	16	(21.3)	3	(8.3)
Treatment Delay (more than one month)	11	(9.9)	6	(8.0)	5	(13.9)
Improper dosage and administration						
No	14	(12.6)	11	(14.7)	3	(8.3)
Yes	97	(87.4)	64	(85.3)	33	(91.7)
Improper regimen (no. of drug and duration)						
No	60	(54.1)	57	(76.0)	3	(8.3)
Yes	51	(45.9)	18	(24.0)	33	(91.7)

	Overall		Drug-resistant pattern			
			MDR-TB		XDR-TB	
	N	(%)	N	(%)	N	(%)
A total episode of MDR-TB Treatment						
1	98	(88.3)	73	(97.3)	25	(69.4)
2	7	(6.3)	2	(2.7)	5	(13.9)
3	4	(3.6)	0	0	4	(11.1)
4	2	(1.8)	0	0	2	(5.6)
Underlying Disease	56	(50.5)	36	(48.0)	20	(55.6)
Diabetes Mellitus	33	(29.7)	23	(30.7)	10	(27.8)
Poor control	18	(16.2)	10	(13.3)	8	(22.2)
Hypertension	17	(15.3)	9	(12.0)	8	(22.2)
Anemia	4	(3.6)	0	0	4	(11.1)
Hepatitis/Cirrhosis	5	(4.5)	2	(2.7)	3	(8.3)
Kidney disease	7	(6.3)	4	(5.3)	3	(8.3)
HIV Infection						
Non-reactive	105	(94.6)	70	(93.3)	35	(97.2)
Reactive	6	(5.4)	5	(6.7)	1	(2.8)
Adverse effect						
Seizure	8	(7.2)	5	(6.7)	3	(8.3)
Optic neuritis	13	(11.7)	10	(13.3)	3	(8.3)
Hearing loss/Vestibular disturbance	39	(35.1)	32	(42.7)	7	(19.4)
Peripheral neuritis	20	(18.0)	17	(22.7)	3	(8.3)
Psychosis	3	(2.7)	3	(4.0)	0	(0.0)

	Overall		Drug-resistant pattern			
			MDR-TB		XDR-TB	
	N	(%)	N	(%)	N	(%)
Depression	7	(6.3)	6	(8.0)	1	(2.8)
Hypothyroid	38	(34.2)	31	(41.3)	7	(19.4)
Arthralgia	47	(42.3)	38	(50.7)	9	(25.0)
Renal Impairment	24	(21.6)	17	(22.7)	7	(19.4)
Nausea/Vomiting	30	(27.0)	21	(28.0)	9	(25.0)
The interim outcome of MDR-TB						
Culture no growth	63	(56.8)	56	(74.7)	7	(19.4)
Culture Growth	12	(10.8)	0	0	12	(33.3)
Culture not done	36	(32.4)	19	(25.3)	17	(47.2)
Outcome of MDR-TB						
Cured	69	(62.2)	65	(86.7)	4	(11.1)
Completed	10	(9.0)	10	(13.3)	0	0
Failed	32	(28.8)	0	0	32	(88.9)

Note: the percentage may not sum up to 100% because of missing value.
Bold letter from table 2-2 to 2-3 means the crude odds ratio of these factors did not include one.

Table 2-3 Source of support regarding drug-resistant pattern (N=111)

			Drug-resistant pattern			
	Overall		MDR-TB		XDR-TB	
	N	(%)	N	(%)	N	(%)
Work while on treatment	56	(50.5)	31	(41.3)	25	(69.4)
Financial Support *	74	(66.7)	47	(62.7)	27	(75.0)
Family Financial support	45	(40.5)	29	(38.7)	16	(44.4)
Community Financial support	2	(1.8)	0	0	2	(5.6)
Healthcare Financial support	33	(29.7)	20	(26.7)	13	(36.1)
Emotional support*	103	(92.8)	72	(96.0)	31	(86.1)
Family Emotional support	97	(87.4)	69	(92.0)	28	(77.8)
Community Emotional support	4	(3.6)	1	(1.3)	3	(8.3)
Healthcare Emotional support	18	(16.2)	9	(12.0)	9	(25.0)
Educational support*	108	(97.3)	74	(98.7)	34	(94.4)
Family Educational support	7	(6.3)	4	(5.3)	3	(8.3)
Healthcare Educational support	106	(95.5)	73	(97.3)	33	(91.7)
Health Care DOT	30	(27.0)	12	(16.0)	18	(50.0)
Only Health Care DOT	15	(13.5)	5	(6.7)	10	(27.8)
Village Health Volunteer DOT	3	(2.7)	2	(2.7)	1	(2.8)
Family DOT	37	(33.3)	30	(40.0)	7	(19.4)
Self-administer	67	(60.4)	46	(61.3)	21	(58.3)
Only self-administration	44	(39.6)	33	(44.0)	11	(30.6)
Difficulty in commute (in MDR treatment episode)	39	(35.1)	25	(33.3)	14	(38.9)
Continue Drinking while treat TB	14	(12.6)	5	(6.7)	9	(25.0)
Harmful alcohol use	47	(42.3)	30	(40.0)	17	(47.2)
Pack Years for cigarette						
No	47	(42.3)	37	(49.3)	10	(27.8)
0.01-5	19	(17.1)	11	(14.7)	8	(22.2)
5.1-15	18	(16.2)	11	(14.7)	7	(19.4)
>15-30	14	(12.6)	11	(14.7)	3	(8.3)

			Drug-resistant pattern			
	Overall		MDR-TB		XDR-TB	
	N	(%)	N	(%)	N	(%)
More than 30	13	(11.7)	5	(6.7)	8	(22.2)
Drug abuse	32	(28.8)	18	(24.0)	14	(38.9)
History of incarcerated	16	(14.4)	14	(18.7)	2	(5.6)

* The proportion from family, community, and healthcare are not mutually exclusive.
Bold letter from table 2-2 to 2-3 means the crude odds ratio of these factors did not include one.

Table 2-4 Effect of biopsychosocial factors which associate with XDR-TB development

Exposure	Crude OR	LL	UL	2.5 %tile	97.5% tile	P-value *	MOR***	LL***	UL***
Age						.0083**			
15-35 vs. 35-55	1.57	0.65	3.80	0.64	4.38	0.32	1.50	0.62	3.65
56-85 vs. 36-55	0.39	0.12	1.34	0.07	1.13	0.14	0.38	0.11	1.33
Gender									
Male vs Female	1.50	0.61	3.67	0.65	4.24	0.37	1.51	0.62	3.70
Non-adherence	10.03	2.98	33.82	3.38	59.20	.0002*	9.27	2.81	30.61
Improper regimen	34.83	9.54	127.23	11.30	>999	<.0001*	31.61	8.76	114.01
Smear positivity	3.03	1.03	8.94	1.23	15.76	0.04*	4.55	1.25	16.57
Adverse effect									
Hearing loss/Vestibular disturbance	0.32	0.13	0.83	0.12	0.72	0.02*	0.37	0.14	0.96
Hypothyroid	0.34	0.13	0.88	0.12	0.84	0.03*	0.4	0.16	1.02
Arthralgia	0.325	0.13	0.78	0.13	0.71	0.01*	0.38	0.15	0.93
SES									
Middle vs Poor*	2.68	0.93	7.77	0.80	8.27	0.07	2.54	0.90	7.12
Rich vs Poor	1.43	0.52	3.92	0.40	3.84	0.49	1.44	0.52	3.99
Work while treatment	3.23	1.39	7.51	1.52	8.33	.0066*	3.52	1.45	8.58
Healthcare DOT	5.25	2.14	12.90	2.08	12.85	.0003*	5.34	2.14	13.31
Only Healthcare DOT	5.38	1.68	17.25	1.57	24.66	.0046*	5.50	1.70	17.76
Family DOT	0.36	0.14	0.93	0.14	0.80	0.04*	0.35	0.13	0.92
Family emotional support	0.30	0.10	0.96	0.17	0.96	0.04*	0.3	0.09	0.95
Harmful alcohol use	1.34	0.60	2.99	0.65	3.05	0.47	1.33	0.60	2.97
Continued alcoholic drinking	5.31	1.59	17.72	1.89	24.38	.0066*	5.17	1.54	17.37

*P-value from Univariate analysis logistic regression.
** P-value of age as a continuous value from the Wilcoxon two-sample test.
*** Conditional logistic regression: matched by region.
OR: Odds ratio
LL: Lower limit of the confidence interval

UL: Upper limit of the confidence interval
MOR: Odds ratio from conditional logistic regression matched by region

Table 2-5 Adjusted effect of biopsychosocial factors which associate with XDR-TB

	Model1			Model 2		
Exposure	AOR	95%CI		AOR	95%CI	
Non-adherence	12.45	1.86	83.36	9.91	1.46	67.28
Improper regimen	28.81	6.59	125.94	35.17	7.47	165.60
Age	0.96	0.91	1.00	0.94	0.89	0.99
Gender						
Male vs. Female				4.85	0.86	27.31
SES						
Middle vs. Poor	1.61	0.26	10.16	1.84	0.27	12.53
Rich vs. Poor	1.37	0.30	6.29	1.48	0.31	7.04
Only healthcare DOT	10.34	1.25	85.55	8.54	1.04	70.33

	Model 3			Model 4 *		
Exposure	AOR	95%CI		AOR	95%CI	
Non-adherence	8.94	1.29	62.21	10.08	1.36	74.87
Improper regimen	40.11	8.82	182.42	37.73	8.09	175.93
Age	0.94	0.89	1.00	0.94	0.88	0.99
Gender						
Male vs. Female	3.37	0.61	18.61	4.85	0.78	30.26
SES						
Middle vs. Poor	2.11	0.36	12.30	2.12	0.39	11.57
Rich vs. Poor	1.28	0.27	6.06	1.40	0.27	7.20
Only healthcare DOT	8.02	1.14	56.42	7.90	1.05	59.75
Harmful alcoholic drink	0.99	0.25	3.92	0.82	0.20	3.41

* Model 4 matched by region, AOR: Adjusted odds ratio

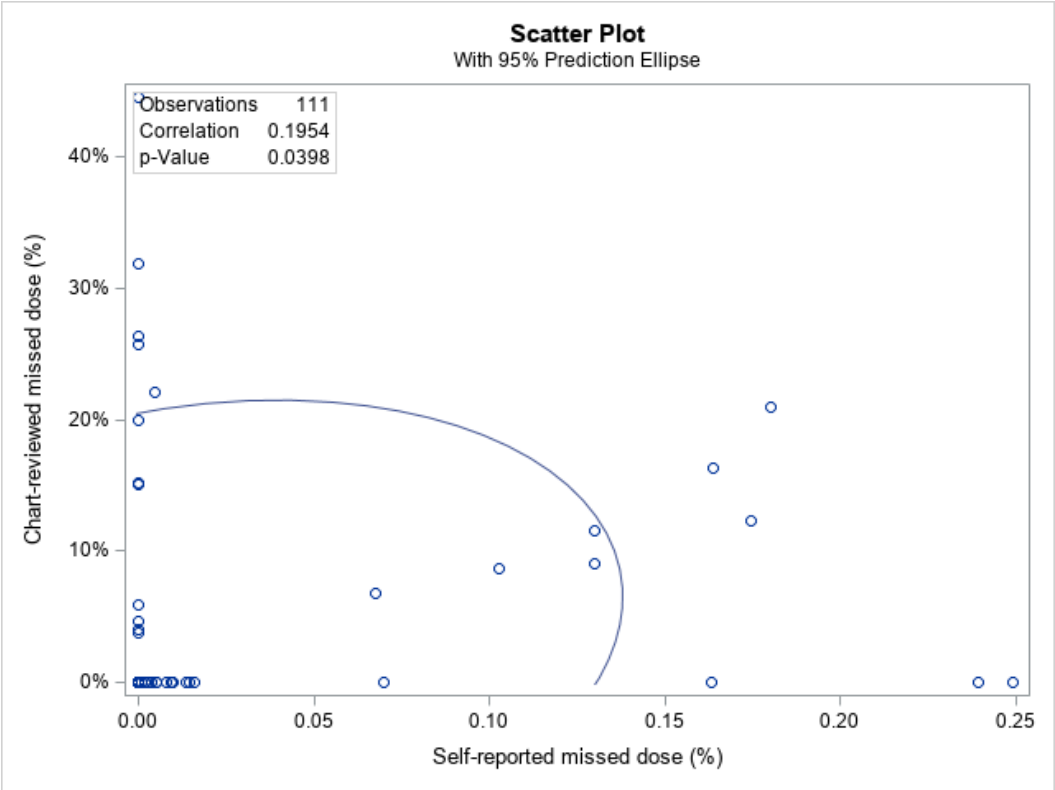

Figure 2-1 Correlation between self-report and chart-reviews missed dose proportion (N=111). The correlation coefficient between the chart-reviewed and the self-reported missed dose is 0.20, which shows weak correlation.

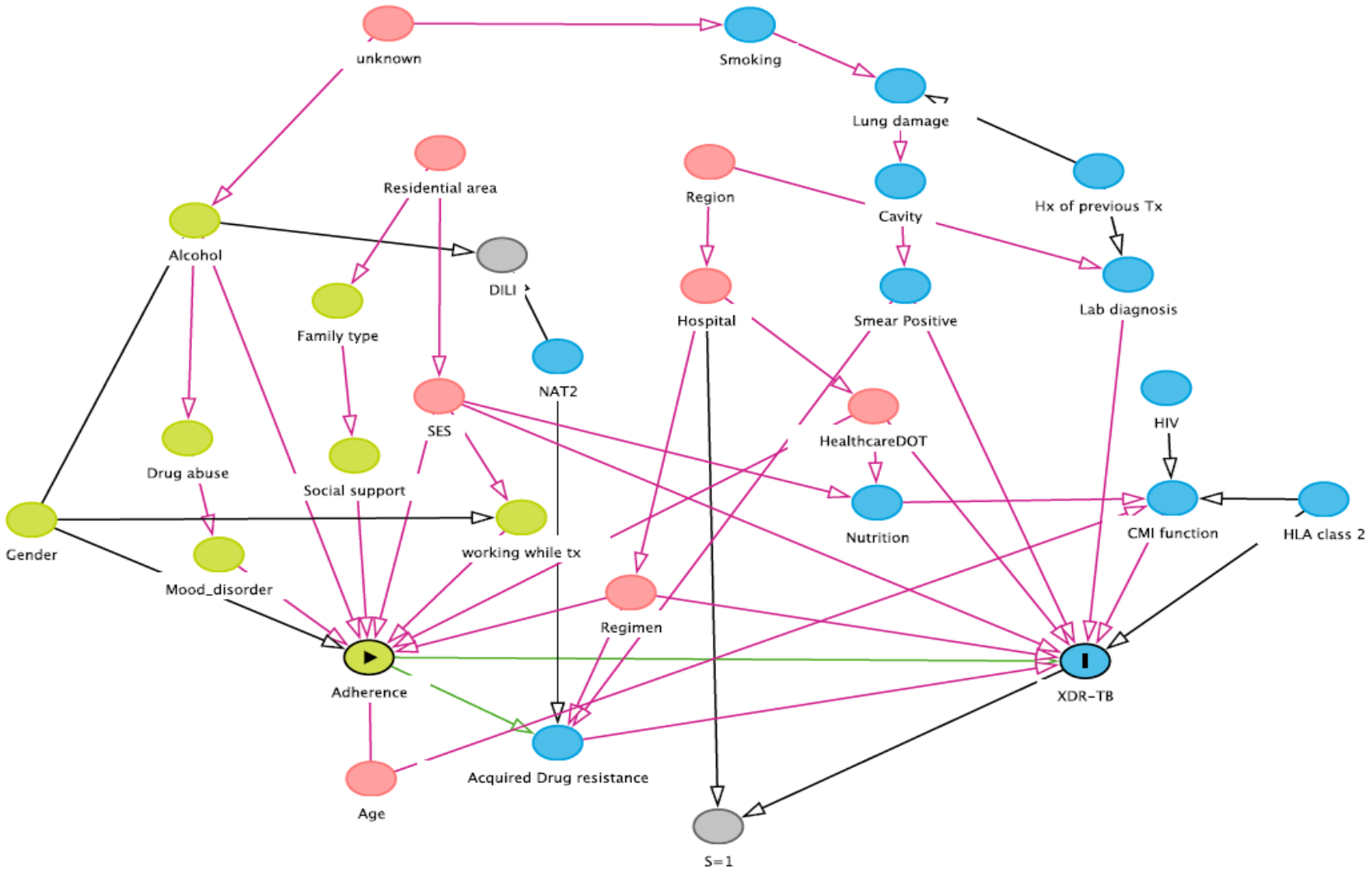

Figure 2-2 Directed Acyclic Graph for biopsychosocial factors associated with XDR-TB development

Chapter 3. The relationship between NAT2 and HLA class2 in cases with XDR-TB

3.1 Abstract

Introduction: Genetic components are believed to be part of the development of TB disease. NAT2 has been identified as a drug-metabolizing enzyme. Identification of human *NAT2* polymorphisms is therefore very significant for predicting the different effective therapeutic doses of INH in fast and slow acetylators. Human leukocyte antigen (HLA) class II molecules play vital roles in the interaction between the host immune system and TB germs by presenting antigenic peptides to helper T-cells. A few studies have explored the association between genetic factors and the XDR-TB pattern. This study aimed to determine the frequencies of HLA class 2 and NAT2 alleles in the Drug-resistant TB groups (both XDR-TB and MDR-TB) and assess the potential association between the polymorphism of NAT2 and HLA class 2 with the susceptibility to XDR-TB

Methods: Blood specimens from 36 secondary XDR-TB cases and 75 MDR-TB controls, which came from north, northeast, south, west, east, and central regions, were sent to the Genomics medical center, Minister of Public Health. NAT 2 haplotype was determined using the Haplotype-specific polymerase chain reactions. HLA class2, comprising HLA-DRB1 and HLA-DQB1, were tested through polymerase chain reaction sequence-specific oligonucleotide (PCR-SSO). All associations were carried out using fisher's exact test and conditional logistic regression in four different genetic models (allelic, carrier, heterozygote, and homozygote).

Results: The *NAT2* haplotypes found by HS-PCR in this study revealed that the six haplotypes (from highest to lowest frequency) were *NAT2*4* (38.7%), *NAT2*6A* (27.9%), *NAT2*7B* (19.4%), *NAT2*5B* (12.6%), *NAT2*12B* (0.5%), and *NAT2*13A* (0.9%). There was an association between *NAT2*4* /5B and XDR-TB disease (OR 3.9, 95%CI 1.2 13.0). However, no significant association between type of NAT2 acetylator and risk for XDR-TB development. For HLA class 2, *HLA*

*DRB1*16:02* heterozygosity was found associated with XDR-TB disease (OR 3.7, 95%CI 1.2, 11.9). No association was detected for any allelic, heterozygote, and homozygote pattern in HLA DQB1.

Conclusion: No significant association between type of NAT2 acetylator and risk for XDR-TB development was found. For HLA class 2, The heterozygosity of *HLA-DRB1*16:02*, which is related to acquired immune deficiency, was found a significant association to cases with XDR-TB. Further research may study larger groups and involve drug-susceptible TB as a control group.

3.2 Introduction

Tuberculosis (TB) is one of the top ten causes of death and the leading cause of death by a single infectious agent (above HIV/AIDS) (8). Nearly one-third of the world's population is believed to be affected by *M. tuberculosis* infection. Nevertheless, only five to 10% of the population develops active TB disease during their lifetime (60). Most individuals possess an immune response that can contain or eliminate the bacteria. Moreover, genetic components are believed to be part of the development of TB disease. For example, twin studies showed a higher concordance rate among monozygotic than dizygotic twins, representing a genetic component to TB vulnerability (61).

Candidate gene approach and genome-wide association studies (GWASs) have shown that several candidate genes such as ASAP1, CD209, HLA-B, MAFB, SLC11A1 are related to TB (62). Several host genes are also associated with susceptibility to TB caused by specific MTB strains. For example, a polymorphism in TLR-2 was associated with susceptibility to TB caused by the East-Asia/Beijing strain from Vietnam (62). Polymorphism in the NAT2 gene amongst humans has been known to change acetylation activity (63). Some host genes are related to the severity of disease and the course of treatment. HLA class 2 is related to the host immune response and contributes to the severity of disease in mycobacterial infection (64).

First, NAT2 genes in humans functioned for the inactivation of INH through acetylation, which is similar to NAT of MTB (65). NAT2 on human chromosome eight has been identified as a drug-metabolizing enzyme. NAT2 phenotype is influenced by host and other factors, including diet, disease, drug therapy—moreover, NAT2 acetylation polymorphism is also related to cancer predisposition by aromatic amine exposure (66). Each individual carried two haplotypes

(diplotype) in a codominant fashion (67, 68). Analysis of NAT2 alleles or haplotypes has led to the classification of individuals as rapid, slow, ultra-slow, and intermediate acetylators.

Rapid Acetylators (RAs) are individual who have *NAT2*4/*4, NAT2*4/*12A, NAT2*4/*13A, NAT2*12A/*12A, NAT2*12A/*13A*, and *NAT2*13A/*13A*. The slow acetylators (SAs) are individuals with *NAT2*5B/*5B, NAT2*5B/*6A*, and *NAT2*5B/*7B*. The ultra-slow acetylators are individuals who have *NAT2*6A/*6A, NAT2*6A/*7B*, and *NAT2*7B/*7B.* The Intermediate acetylators (IAs) are the combination between fast and slow NAT2 haplotypes such as, *NAT2*4/*5B, NAT2*4/*6A, NAT2*4/*7B, NAT2*5B/*12A, NAT2*5B/*13A, NAT2*6A/*12A, NAT2*6A/*13A, NAT2*7B/*12A*, and *NAT2*7B4/*13A* (67, 68).

Identification of human NAT2 polymorphisms is therefore very significant for predicting the different effective therapeutic doses of INH in fast and slow acetylators (69, 70). As INH is rapidly acetylated and excreted in fast acetylator, it is proposed that the optimal concentration of the drug (INH) is not available in the lungs or affected tissues to mediate its action. As a result of consistently lower serum concentrations of INH and minimal exposure of INH to MTB, gradual buildup of INH resistance occurs. From the host point of view, rapid acetylators may contribute to a certain degree of INH resistance based on NAT2 gene polymorphism (71) and related to poor treatment outcomes (69, 72).

Human leukocyte antigen (HLA) class II molecules play vital roles in the interaction between the host immune system and TB germs by presenting antigenic peptides to helper T-cells (73). Several studies have reported an association between HLA class II genes and TB (74-78). However, most of these studies had insufficient statistical power because of the inclusion of a small number of patients with TB. Vejbaesya et al. revealed that *HLA-DQB1*05:02*, *HLA-*

*DQA1*06:01*, and *HLA-DQB1*03:01* alleles were associated with TB (77). Goldfeld et al. suggested that *HLA-DQB1*05:03* alleles were associated with susceptibility to TB in Cambodian patients (78). In 2010, a systematic review showed the variation in HLA antigen and disease susceptibility in the different ethnic populations (73, 79). Therefore, it is unclear whether variations in HLA class II genes play a role in increasing susceptibility to TB.

The result from the Northwest region in Russia showed that the alleles *DRB1*15* and *DQB1*03* represented the resistant pattern in pulmonary tuberculosis (80). Kuranov et al. found that *HLA-DRB1*08:01* and *DRB1*08:03* alleles were associated with TB progression, the development of drug resistance (MDR-TB), and the recurrence of disease in Kazakhstan (80). Kuranov et al. also stated that *HLA-DQB1*06:01* was found to have linkage positively with *DRB1*08:03*.

Moreover, HLA-DRB1 and HLA-DQB1 were found to be associated with Adult-onset immunodeficiency. The study demonstrated a strong association between HLA-DRB1 and DQB1 alleles, especially *HLA-DRB1*15:01, DRB1*16:02, DQB1*05:01* and *DQB1*05:02*, and disseminated opportunistic infection, such as non-tuberculous mycobacterial disease, with acquired anti-IFN-γ autoantibody in Thais (81, 82).

After reviewing literature about NAT2 and HLA class 2, we hypothesized that slow or fast acetylator type of NAT2 might be found more in XDR-TB cases than MDR-TB controls. In addition, specific polymorphism in HLA class 2 could be associated with XDR-TB by immunodeficiency pathway. Nonetheless, few studies reported the impact of NAT2 and HLA class2 on the development of XDR-TB. This study aimed to assess the potential association between the polymorphism of NAT2 and HLA class 2 with the susceptibility to XDR-TB.

3.3 Methods

Study design and population

We conducted a case-control study and recruited all secondary XDR-TB cases that were reported to the Bureau of Tuberculosis (BTB) from October 2014 to June 2019. The total reported XDR cases in Thailand were 56 cases (Primary: 11 cases, Secondary: 45 cases). Specific for secondary XDR-TB, nine of 45 cases died before we started. A total of 36 secondary XDR-TB cases were enrolled at 32 original hospital sites, across 29 provinces in Thailand. Next, we asked health staff from the TB clinic to randomly select MDR-TB controls from the same hospital where XDR-TB cases were treated, from 2015 to 2018. The proportion of cases to controls was one XDR-TB to two MDR-TB.

Case definition: Secondary XDR-TB cases (36 cases) were all TB patients who had a history of anti-TB treatment for more than one month with culture- or molecular-proven XDR-TB registered in any hospital in Thailand. Specimens from these patients were sent to The National TB Reference Laboratory (NTRL) for second-line drug susceptibility testing. Control definition: MDR-TB in the control group consisted of patients diagnosed with MDR-TB in the fiscal year of 2015-2019 and matched by province or public health region as the case with approximately double the number of controls (75 controls) being enrolled. *Inclusion criteria*: XDR-TB patients registered for the treatment of XDR-TB (cases) patients with MDR-TB (controls), who provided written informed consent. *Exclusion criteria*: age less than 18 years old, prisoner, non-Thai, and refusal to participate.

Eligible TB patients were contacted by the TB coordinators in the selected hospitals either in person or through a telephone call. The participants were informed of the study and had a chance

to decide whether they would participate or not. For the patients who were willing to participate, we abstracted their data from clinical reporting forms that were completed by TB health staff on the appointment date.

After reviewing the health record, it was followed by a patient interview based on the appointment date after patients had visited the TB clinic for their treatment. TB patients provided signed informed consent before the audio-recorded interview started. Trained research assistants conducted the semi-structured interview in Thai, each interview lasting for 30 – 40 minutes. After the interviewing process, patients' venous blood had been drawn for three to five milliliters in EDTA tubes.

Laboratory method: DNA extraction for host genotyping

Blood specimens were sent to Genomics medical center, Minister of Public Health, for NAT 2 haplotype. DNA samples were extracted from EDTA blood (250 ul) using a commercial kit (QIAamp DNA blood mini kit, QIAGEN GmbH, Germany) and quantitated using a spectrophotometer (Nanodrop- 100, Wilmington, DE 19810, USA) . The Haplotype-specific polymerase chain reactions (HS-PCR) for NAT2 diplotyping used six reaction tubes, which each tube containing a specific primer pair for one of the six haplotypes (*NAT2*4, NAT2*5B, NAT2*6A, NAT2*7B, NAT2*12A,* and *NAT2*13A)* that most commonly found in Thai populations

The final PCR (12 µl) was composed of 1X ready mix reagent (KAPA2G Fast Multiplex Mix, KAPA Biosystems, Boston, Massachusetts, USA) , the NAT2- specific primer pair (0. 3 µM) , the *TIMP1* primer pair (0.1 µM), and 20-50 ng of a DNA sample. After denaturation at 95°C for 5 minutes, 35 cycles of amplification (95°C for 20 seconds, 65°C for 20 seconds, and 72°C for 30 seconds) were performed. The six NAT2 haplotypes were directly determined by evaluating the specific sizes of

the PCR products in 1.5% agarose gels stained with ethidium bromide. Each NAT2 haplotype reaction tube produced a specific band when a sample was positive for that particular haplotype, except for the *NAT2*13* reaction tube, which produces two bands of 366 bp and 641 bp from one forward primer and two reverse primers (68).

HLA typing using bead-based methods

HLA class II alleles, comprising HLA-DRB1, and HLA-DQB1 were genotyped using sequence-specific oligonucleotides (PCR-SSOs). The DNA samples were amplified by polymerase chain reaction (PCR). The PCR products were hybridized against a panel of SSO probes on coated polystyrene microspheres, that had sequences complementary to the stretches of polymorphism within the target HLA class II alleles, using commercial kits (WAKFlow HLA Typing kit HLA-DRB1 and HLA-DQB1, Wakunaga Pharmaceutical Co., Ltd. Hiroshima, Japan). The amplicon-probe complex was then visualized using a colorimetric reaction and fluorescence detection technology by the Luminex®IS 100 system (Luminex Corporation, Austin, Texas, USA). Interpretations of HLA class II alleles from the probe signals were performed using WAKFlow® typing software (Wakunaga Pharmaceutical Co., Ltd. Hiroshima, Japan)

The technicians performing the genotyping were blinded to case and control status. All study subjects were provided oral and written informed consent

Data analysis

Data from the case record form were entered into the data entry form. Data entry used a double data entry method in Excel (Microsoft Corp., Seattle, WA), then imported and analyzed in SAS version 9.4 (SAS Institute, Inc., Cary, NC). Medians with interquartile ranges (IQRs) were calculated for continuous variables, and frequency distributions were tabulated for categorical

variables. Groups were compared using the two-sample Wilcoxon-Mann-Whitney test for continuous data and Chi-square/Fisher's exact test for categorical data.

All associations were carried out using fisher's exact test in four different genetic models (allelic, carrier, heterozygote, homozygote). To evaluate odds ratio (OR) with 95% confidence interval (95%CI) for the susceptibility to develop XDR-TB, haplotype and diplotype frequencies were compared between TB cases who did or did not develop XDR-TB, using conditional logistic regression analysis.

Ethics statement

The Institutional Review Board of the University of California Los Angeles (UCLA IRB#19-000158) and the Ethics Committee for Research in Human Subjects Department of Diseases Control (FWA #00013622) approved this study in 2019. This study was also approved by ten local Institutional reviewed boards from selected hospitals. In addition, 22 hospitals approved the informed consent forms before we conducted the study in their hospitals.

3.4 Results

We researched in order to assess the different patterns in the allelic pattern of NAT2 and HLA class 2 among XDR-TB cases and MDR-TB control.

3.4.1 Demographic pattern and clinical characteristics by drug-resistant pattern

Overall, 36 case participants were later diagnosed with XDR-TB, and 75 control participants did not develop XDR-TB disease (table 3-1). There was a total of 111 subjects aged between 18-82 years. The median age of MDR-TB controls was 47 years (interquartile range IQR [34-59]), while the median age of XDR-TB cases was 40 years (IQR [28-52]). About 50% of participants had underlying diseases. Six out of 111 cases were PLHIV (one case or 2.8% in the XDR-TB group).

In the aspect of treatment, 91.3% of the group who later developed XDR-TB received improper regimen and improper dosage and administration; whereas, 24% of cases with MDR-TB who did not develop XDR-TB received improper regimen, and 85 % received improper dosage and administration. Only two cases (5.6%) in the group who later developed XDR-TB experienced hepatitis while on MDR-TB treatment and just three cases (4%) in the MDR-TB controls. In four cases in the XDR-TB group who were cured on their previous TB treatment, two of them have developed XDR-TB one and five years later, respectively.

3.4.2 Direct NAT2 diplotyping by HS-PCR

The NAT2 haplotypes found by HS-PCR in this study revealed that the six haplotypes (from highest to lowest frequency) were *NAT2*4* (38.7%), *NAT2*6A* (27.9%), *NAT2*7B* (19.4%), *NAT2*5B* (12.6%), *NAT2*12B* (0.5%), and *NAT2*13A* (0.9%) (table 3-2). Each individual carried two haplotypes (diplotype) in a codominant fashion. Corresponding to the frequency of NAT2 diplotype, individuals with *NAT2*4/6A* (19.8%), *NAT2*4/7B* (16.2%), and *NAT2*4/*4* (14.4%) were the first three most common diplotypes in this study (table 3-3).

*NAT2*4/5B* (intermediate acetylator) was believed to be associated with the cases with XDR-TB (OR 3.91, 95%CL 1.18, 13.00). No significant association between type of NAT2 acetylator and risk for XDR-TB development was found. In five cases who experienced the adverse event of hepatitis, three out of five were ultra-slow acetylators (*NAT2*6A/*7B*). Because of a few cases with an adverse event of hepatitis, we could not find an association between acetylator type and drug-induced liver injury.

3.4.3 Evaluate the relationship between HLA-DRB1, HLA-DQB1, and XDR-TB

Table 3-5 shows the distribution of the DRB1 alleles. None of these alleles and homozygote patterns demonstrated frequencies that were significantly different between the cases who did and did not develop XDR-TB. Nonetheless, the heterozygosity of *HLA-DRB1*16:02* was found more in cases with XDR-TB (OR 3.71, 95% CL 1.15, 11.89). Furthermore, table 3-6 shows no association was detected for any alleles, heterozygote, or homozygote pattern in HLA DQB1.

3.5 Discussion

In this study, the participants from all regions of Thailand showed the six haplotypes (*4, 6A, and 7B at a high frequency; *5B, 12A, and 13A at a low frequency). They had the same distribution of NAT 2 haplotype as the previous report in 650 samples randomly selected from a nationwide Thailand population study by Wichuckchinda et al. (68). In the aspect of acetylators, the distribution of acetylators in our population was similar to the distribution of NAT2 acetylator in Thailand (n=650), which was also from the Third Thailand National Health Examination Survey Programs in the study by Wichuckchinda et al. (68).

The result of diplotype analyzed cooperating with the development of XDR-TB exhibited the association of *NAT2*4/*5B*, which was found approximately four times more in the cases with XDR-TB. However, *NAT2*4/5B* is an intermediate type acetylation, and we could not find the biological explanation to support this result. Moreover, there was no significant association between NAT2 acetylator types and XDR-TB disease.

Our study showed that *DRB1*16:02* in the heterozygote pattern was more frequent among patients with later developed to XDR-TB than the control who did not develop. Previous studies (81, 82) also reported that *HLA-DRB1*16:02* and *DQB1*15:02* are associated with the clinical

syndrome of disseminated nontuberculous mycobacterial (NTM) diseases. This clinical syndrome has been known association with an acquired autoantibody to interferon-gamma (IFN-γ)(83) and responded poorly to antimycobacterial therapy, despite proper drug administration and good compliance (84).

Our study had three main limitations. First, there were only 36 secondary XDR-TB cases in this study. Therefore, it was too few for the genetic study; however, we still found the association between *HLA-DRB1*16:02* and XDR-TB disease. Second, we do not have the result of autoantibody to IFN-γ to support this finding. Third, if we correct for multiple comparisons by controlling the false discovery rate (Benjamini-Hochberg procedure), we will not find any association between NAT2, HLA class 2, and XDR-TB disease. Therefore, further research about *HLA-DRB1*16:02* and autoantibody testing for IFNγ may involve a larger group and include drug-susceptible TB as a control group.

3.6 Tables and figures

Table 3-1 Demographic pattern and clinical characteristics by drug-resistant patterns (N=111)

			Drug-resistant pattern			
	Overall		MDR-TB		XDR-TB	
	N	(%)	N	(%)	N	(%)
Age group (years)at diagnosis						
15-35	37	(33.3)	21	(28.0)	16	(44.4)
36-55	49	(44.1)	33	(44.0)	16	(44.4)
56-85	25	(22.5)	21	(28.0)	4	(11.1)
Age (median, IQR) at diagnosis	42 (32, 55)		47 (34,59)		40 (28, 52)	
Gender						
Female	34	(30.6)	25	(33.3)	9	(25.0)
Male	77	(69.4)	50	(66.7)	27	(75.0)
BMI Category						
Underweight	53	(47.7)	33	(44.0)	20	(55.6)
Optimal	41	(36.9)	29	(38.7)	12	(33.3)
Overweight	10	(9.0)	7	(9.3)	3	(8.3)
Obese	7	(6.3)	6	(8.0)	1	(2.8)
Region						
Central	34	(30.6)	23	(30.7)	11	(30.6)
East	12	(10.8)	8	(10.7)	4	(11.1)
North	9	(8.1)	6	(8.0)	3	(8.3)
Northeastern	20	(18.0)	14	(18.7)	6	(16.7)

	Overall		Drug-resistant pattern			
			MDR-TB		XDR-TB	
	N	(%)	N	(%)	N	(%)
South	21	(18.9)	14	(18.7)	7	(19.4)
West	15	(13.5)	10	(13.3)	5	(13.9)
Underlying Disease	56	(50.5)	36	(48.0)	20	(55.6)
Diabetes Mellitus	33	(29.7)	23	(30.7)	10	(27.8)
Poor control	18	(16.2)	10	(13.3)	8	(22.2)
Hypertension	17	(15.3)	9	(12.0)	8	(22.2)
Hepatitis/Cirrhosis	5	(4.5)	2	(2.7)	3	(8.3)
Kidney disease	7	(6.3)	4	(5.3)	3	(8.3)
HIV Infection						
Non-reactive	105	(94.6)	70	(93.3)	35	(97.2)
Reactive	6	(5.4)	5	(6.7)	1	(2.8)
Smear positivity						
NA	16	(14.4)	11	(14.7)	5	(13.9)
Neg	29	(26.1)	23	(30.7)	6	(16.7)
Scanty-1+	26	(23.4)	16	(21.3)	10	(27.8)
2+-3+	40	(36.0)	25	(33.3)	15	(41.7)
Site of TB						
Pulmonary	105	(94.6)	70	(93.3)	35	(97.2)
Both pulmonary & extrapulmonary	6	(5.4)	5	(6.7)	1	(2.8)
Cavity	55	(49.5)	33	(44.0)	22	(61.1)

			Drug-resistant pattern			
	Overall		MDR-TB		XDR-TB	
	N	(%)	N	(%)	N	(%)
Improper dosage & administration						
No	14	(12.6)	11	(14.7)	3	(8.3)
Yes	97	(87.4)	64	(85.3)	33	(91.7)
Improper regimen (no. of drug and duration)						
No	60	(54.1)	57	(76.0)	3	(8.3)
Yes	51	(45.9)	18	(24.0)	33	(91.7)
Adverse effect: Hepatitis	5	(4.5)	3	(4.0)	2	(5.6)
Interim outcome of MDR-TB						
Culture no growth	63	(56.8)	56	(74.7)	7	(19.4)
Culture Growth	12	(10.8)	0	0	12	(33.3)
Culture not done	36	(32.4)	19	(25.3)	17	(47.2)
Final outcome of MDR-TB						
Cured	69	(62.2)	65	(86.7)	4	(11.1)
Completed	10	(9.0)	10	(13.3)	0	0
Failed	32	(28.8)	0	0	32	(88.9)

Table 3-2 NAT2 Allele frequencies in this study

	Overall		Case		Control	
NAT2 alleles	n	%	n	%	n	%
***4**	86	38.7	32	44.4	54	36.0
***5B**	28	12.6	9	12.5	19	12.7
***6A**	62	27.9	17	23.6	45	30.0
***7B**	43	19.4	14	19.4	29	19.3
***12A**	1	0.5	0	0.0	1	0.7
***13A**	2	0.9	0	0.0	2	1.3
	222	100				

Table 3-3 The association between NAT2 genotype and the development to XDR-TB (N=111)

NAT2	XDRvsMDR					
	case (n.XDR=36)		control (n.MDR=75)		OR	(95% CI)
genotypic.name	count	freq	count	freq		
*4/*13A	0	0.0000	1	0.0133	0.00	(0.00 - 999.99)
*4/*4	8	0.2222	8	0.1067	2.35	(0.82 - 6.72)
***4/*5B**	**8**	**0.2222**	**5**	**0.0667**	**3.91**	**(1.18 - 13.00)**
*4/*6A	4	0.1111	18	0.2400	0.38	(0.12 - 1.25)
*4/*7B	4	0.1111	14	0.1867	0.54	(0.16 - 1.75)
*5B/*13A	0	0.0000	1	0.0133	0.00	(0.00 - 999.99)
*5B/*5B	0	0.0000	1	0.0133	0.00	(0.00 - 999.99)
*6A/*12A	0	0.0000	1	0.0133	0.00	(0.00 - 999.99)
*6A/*5B	1	0.0278	5	0.0667	0.40	(0.05 - 3.52)
*6A/*6A	3	0.0833	7	0.0933	0.90	(0.22 - 3.68)
*6A/*7B	6	0.1667	7	0.0933	1.98	(0.60 - 6.53)
*7B/*5B	0	0.0000	6	0.0800	0.00	(0.00 - 999.99)
*7B/*7B	2	0.0556	1	0.0133	4.42	(0.37 - 52.85)
Total	36	1.0000	75	1.0000		

Table 3-4 Distribution of NAT2 acetylator status in the study participants (N=111)

	Drug-resistant pattern								
	Overall		XDR-TB		MDR-TB		OR	(95%CI)	
	N	(%)	N	(%)	N	(%)			
Acetylator type									
Rapid	17	(15.3)	8	(22.2)	9	(12.0)	2.21	0.74	6.64
Intermediate	55	(49.5)	16	(44.4)	39	(52.0)	1		
Slow	13	(11.7)	1	(2.8)	12	(16.0)	0.21	0.03	1.76
Ultraslow	26	(23.4)	11	(30.6)	15	(20.0)	1.81	0.68	4.85

Table 3-5 The association between DRB1 alleles and the development of XDR-TB

DRB1	Allelic			Heterozygote				
	Case	Control	P-value	Case	Control	OR	95%CI	
0101	0	1	1.00	0	1	0.00	0.00	999.99
0301	4	11	0.78	4	7	1.23	0.34	4.47
0401	1	1	0.54	1	1	1.96	0.12	32.80
0403	1	1	0.54	1	1	2.16	0.14	34.61
0404	0	1	1.00	0	1	0.00	0.00	999.99
0405	5	12	1.00	5	12	0.88	0.29	2.69
0406	2	1	0.25	2	1	4.00	0.36	45.20
0701	4	13	0.59	4	11	0.71	0.21	2.41
0803	1	3	1.00	1	3	0.66	0.06	7.16
0901	7	22	0.40	3	18	0.30	0.08	1.09
1001	1	8	0.28	1	8	0.24	0.03	2.01
1101	1	1	0.54	1	1	2.12	0.13	33.92
1106	2	1	0.25	2	1	4.00	0.36	45.20
1152	1	2	1.00	1	2	1.05	0.10	11.62
1201	0	3	0.55	0	3	0.00	0.00	999.99
1202	14	23	0.45	12	21	1.26	0.53	2.96
1219	0	1	1.00	0	1	0.00	0.00	999.99
1301	1	0	0.32	1	0	999.99	0.001	999.99
1302	1	4	1.00	1	4	0.49	0.05	4.53
1401	4	9	1.00	4	9	0.92	0.26	3.22
1404	1	5	0.67	1	5	0.42	0.05	3.66
1405	1	1	0.54	1	1	2.00	0.13	31.98
1410	2	0	0.10	2	0	999.99	0.001	999.99
1415	1	0	0.32	1	0	999.99	0.001	999.99
1501	2	5	1.00	2	5	0.86	0.16	4.60
1502	7	16	1.00	7	16	0.88	0.33	2.35
1602	**8**	5	**0.03**	**8**	**5**	**3.71**	**1.15**	**11.89**
Total	72	150						

Table 3-6 The association between DQB1 alleles and the development of XDR-TB

DQB1	Allelic			Heterozygote				
	Case	Control	P-value	Case	Control	OR	95%CI	
0201	4	12	0.59	4	8	1.07	0.30	3.77
0202	4	10	1.00	4	10	0.80	0.23	2.73
0301	12	21	0.69	8	17	0.94	0.36	2.48
0302	7	11	0.60	5	11	0.93	0.29	3.00
0303	5	16	0.47	5	16	0.60	0.20	1.79
0328	1	2	1.00	1	2	1.05	0.10	11.62
0401	4	8	1.00	4	8	1.05	0.31	3.66
0402	0	2	1.00	0	2	0.00	0.00	999.999
0501	5	20	0.18	5	18	0.50	0.17	1.50
0502	21	25	0.04	13	19	1.70	0.71	4.06
0503	4	7	0.75	4	7	1.23	0.35	4.39
0601	2	11	0.23	2	9	0.46	0.10	2.21
0602	1	1	0.54	1	1	2.24	0.11	44.88
0603	1	0	0.32	1	0	999.999	0.00	999.999
0609	0	3	0.55	0	3	0.00	0.00	999.999
0625	1	1	0.54	1	1	1.96	0.12	31.31
Total	72	150						

Chapter 4. Sputum culture conversion as a prognostic marker for the development of XDR-TB

4.1 Abstract

Introduction: Proper treatment and enhanced case detection are needed to ensure a success rate and stop spreading infection. Monthly sputum culture is considered as a proxy for treatment effectiveness. Knowing the time to sputum culture conversion is often used as a prognostic marker for the treatment outcome in pulmonary TB and possibly the development of XDR-TB. However, few studies in Thailand reported time to sputum culture conversion and the development of XDR-TB, and pattern of reversion in MDR-TB treatment episode. In this study, we explored factors that related to sputum culture conversion and demonstrated the pattern of time to conversion in MDR-TB treatment episode who did and did not develop XDR-TB. We also identify the optimal timepoints for the initial sputum culture conversion that could be considered as a marker for XDR-TB development in the context of Thailand.

Methods: A total of 38 secondary XDR-TB cases and 76 MDR-TB controls were enrolled at 32 original hospital sites, across 29 provinces in Thailand. Time to sputum culture conversion was presented in Kaplan-Meier survival estimates. We used the Log-rank test and Cox Proportional Hazards Regression to find the difference of sputum culture conversion among XDR-TB during MDR-TB treatment was given and MDR-TB control and to estimate for the effect of each variable on initial culture conversion, respectively. Receiver operating characteristic curves were done to see the effect of different time points for sputum culture conversion on balance between sensitivity and specificity and determine the optimal point using the Youden's index.

Results: Of 38 cases who later developed XDR-TB, 26 (68.4%) had initial sputum culture conversion in a median of 3.5 months, but 1.5 months for the MDR-TB group who did not. In multiple Cox Proportional Hazards Regression, the improper regimen was found to significantly

reduce the chance of culture conversion (aHR 0.24, 95%CL 0.15, 0.38) controlling for the wrong prescription for dosage and administration, and follow-up sputum culture according to guidelines. Lack of culture conversion at four-month had the maximum Youden's index and can be the optimal point to predict the chance of failure in treatment and develop XDR-TB. In 102 converters, 24 (20.2%) experienced subsequent positive culture or reversion. The 20 cases did develop to XDR-TB, but four did not.

Conclusion: Our study showed that an effective regimen and monthly follow-up sputum culture should be provided to ensure the culture conversion of MDR-TB treatment. Moreover, sputum culture conversion at four-month could be a useful prognostic marker for the development of XDR-TB and the failure outcome.

4.2 Introduction

Tuberculosis (TB) is one of the top ten causes of death by a single infectious agent (above HIV/AIDS) (8). Multidrug-resistant TB (MDR-TB), defined as TB resistant to at least INH and RIF, is an increasing global health threat (8). According to the 2019 global report, the number of cases starting MDR-TB treatment in 2018 was equivalent to 84% of the 186,772 MDR/RR-TB patients notified in 2018. The proportion of MDR/RR-TB patients in the 2016 cohort who completed treatment was 56% (8). Specifically, in Thailand, which is in one of the high MDR-TB burden countries, the number of cases starting MDR-TB treatment in 2018 was equivalent to 910 (69.36%) of the 1,312 MDR/RR-TB patients notified in 2018. The proportion of MDR/RR-TB patients in the 2016 cohort who completed treatment was 61%(8).

Proper treatment and enhanced case detection are needed to ensure a success rate and stop spreading infection. The total treatment of MDR-TB takes at least 20 months, and a minimum total length of treatment of 18 months after culture conversion was suggested for most patients (17). Monthly sputum culture is considered as a proxy for treatment effectiveness (43). Knowing the time to sputum culture conversion (SCC), defined as "two consecutive negative cultures taken at least 30 days apart following an initial positive culture" (17), is often used as a prognostic marker for the treatment outcome in pulmonary TB (85) and possibly the development of XDR-TB.

Non-conversion of sputum culture at the end of the intensive phase of treatment tends to yield unfavorable results, especially failure and death (85, 86). Moreover, a delay after a four-month conversion was considered as a precondition for suspecting MDR-TB treatment failure (85). Although the SCC is significant in MDR-TB treatment, we found only one study (85) about sputum culture conversion in pulmonary MDR-TB, which included Thailand in their patient populations,

"The Preserving Effective TB Treatment Study (PETTS)." This study by Kurbatova et al. showed that time to sputum culture conversion converted at six-month and two-month, could be considered as a surrogate marker for treatment outcome in patients with MDR-TB. However, the conversion at six-month was more substantial in outcome prediction than two-month (85).

Earlier detection of the patient's nonresponse to MDR-TB treatment may allow doctors to adjust therapy and avoid unfavorable outcomes. However, few studies reported time to sputum culture conversion and the development of XDR-TB. Therefore, this study was conducted to demonstrate the pattern of time to conversion in MDR-TB treatment episode who did and did not develop XDR-TB and evaluate factors that affected the chance of culture conversion. Moreover, we identify the optimal timepoints for the initial sputum culture conversion that could be considered as a marker for XDR-TB development in the context of Thailand.

4.3 Methods

Study design and population

We conducted a matched case-control study and recruited all secondary XDR-TB cases that were reported to the Bureau of Tuberculosis (BTB) from October 2014 to June 2019. The total reported XDR cases in Thailand were 56 cases (Primary: 11 cases, Secondary: 45 cases). Specific for secondary XDR-TB, seven of 45 cases did not have the report of sputum culture. A total of 38 secondary XDR-TB cases were enrolled at 32 original hospital sites, across 29 provinces in Thailand. Next, we asked health staff from the TB clinic to randomly select MDR-TB controls from the same hospital where XDR-TB cases were treated, from 2015 to 2018. The proportion of cases to controls was one XDR-TB to two MDR-TB.

Case definition: Secondary XDR-TB cases (38 cases) were all TB patients who had a history of anti-TB treatment for more than one month with culture- or molecular-proven XDR-TB registered in any hospital in Thailand. Specimens from these patients were sent to The National TB Reference Laboratory (NTRL) for second-line drug susceptibility testing. Control definition: MDR-TB in the control group consisted of patients diagnosed with MDR-TB in the fiscal year of 2015-2019 and matched by province or public health region as the case, with double the number of controls (76 controls) being enrolled.

Inclusion criteria: XDR-TB patients registered for the treatment of XDR-TB (cases) patients with MDR-TB (controls), who provided written informed consent. *Exclusion criteria*: age less than 18 years old, prisoner, non-Thai, and refusal to participate.

With the total sample size of 114 (38 XDR-TB cases and 76 MDR-TB controls), this study has about 80% power to detect a difference in time to sputum culture conversion while on MDR-TB treatment between secondary XDR-TB cases and MDR-TB controls. The power of this study was calculated based on using a 1:2 matched design by geographical area, with a 95% confidence interval (two-sided), and alpha at 0.05. For the comparison of the survival pattern in both groups, the median sputum culture conversion time of patients with successful treatment outcomes was 92 days (95% CI 85–99 days), which was much shorter ($p<0.001$) than for the patients with treatment failure or death (174 days, 95% CI 0–513)(87). The estimated number of XDR cases needed to achieve 80% power in the present study is 38. We performed sample size calculations using STATA 15 (StataCorp, College Station, TX, USA).

Data collection and tools

The case record form was developed from the XDR-TB request forms, which are included in the guidelines for prevention and control of Extensively Drug-Resistant TB in Thailand under the Communicable Disease Act of 2015 (11). This form is used to collect socio-demographic, clinical, and laboratory data from the medical charts of patients. **We collected all the data while both groups of patients were in the MDR-TB treatment phase.**

Eligible TB patients were contacted by the TB coordinators in the selected hospitals either in person or through a telephone call. The participants were informed of the study and had a chance to decide whether they would participate or not. For the patients who were willing to participate, we abstracted their data from clinical reporting forms that were completed by TB health staff on the appointment date. For the XDR-TB cases who died, the director of those hospitals approved informed-consent instead. Moreover, we completed the case record forms by retrospectively reviewing electronic health records, medical charts, and laboratory databases.

Operational definition and classification (17, 42, 43, 88)

Sputum culture conversion: Two or more consecutive negative cultures from sputum samples obtained at least 30 days apart. Two negative cultures in sequence counted towards this definition even if there were missing cultures between these results. We defined time to initial sputum culture conversion as the time in months from the date of the start of MDR-tuberculosis treatment to the date of specimen collection for the first of these two consecutive negative cultures.

Sustained sputum culture conversion: An absence of any subsequent positive cultures after conversion.

Sputum culture reversion: At least one subsequent positive culture after the initial conversion.

Persistent culture positivity: No culture conversion in patients with a baseline positive culture. The clinical sites assigned the treatment outcomes.

Follow-up sputum: Smear was sent for every month of treatment; culture was sent for every month in the intensive phase, and every two months in the continuation phase (17).

Cure: At least three consecutive negative cultures in the continuation phase of treatment, obtained at least 30 days apart (17).

Treatment completed: Successful completion of treatment with fewer than three cultures done in the continuation phase of treatment (17).

Failure: Stop MDR-TB treatment or necessary to add two more anti-TB drugs (17).

Default or loss to follow-up: Patients not taking anti-TB drugs or not visiting TB clinic for two months or more consecutively after starting treatment (17).

Treatment success: Cure or completion of treatment. The poor outcome: failure or death of any cause during MDR-TB treatment.

Improper regimen: Insufficient number of medications or shorter duration of treatment than it was recommended. An insufficient number of drugs defined as the intensive phase consists of less

than four effective second-line anti-TB drugs or if the continuation phase consists of less than three effective drugs.

Improper administration and dosage: *Anti-TB drugs* are not prescribed in the normal dosage range or proper interval to achieve the peak concentration with a minimal side effect.

Duration: A 6-8-month intensive phase with at least four months past culture conversion was recommended as the treatment duration for most patients. 20-month total treatment duration with at least 18 months after culture conversion was recommended as the total duration of treatment for most patients (17, 42).

Sensitivity: The proportion of patients with a lack of sputum culture conversion by the beginning to 15 months of the treatment among those with XDR-TB or failure treatment outcomes.

Specificity: The proportion of patients with sputum culture conversion by the beginning to 15 months of the treatment among those who did not develop XDR-TB or successful outcome.

Data analysis

Data from the case record form were entered into the data entry form. Data entry used a double data entry method in Excel (Microsoft Corp., Seattle, WA), then imported and analyzed in SAS version 9.4 (SAS Institute, Inc., Cary, NC). Medians with interquartile ranges (IQRs) were calculated for continuous variables, and frequency distributions were tabulated for categorical variables. Groups were compared using the two-sample Wilcoxon-Mann-Whitney test for continuous data.

Time to an event (Time to sputum culture conversion) was presented in Kaplan-Meier survival estimates. It used the Log-rank test to find the difference of time to event among XDR-TB while MDR-TB treatment was given and MDR-TB control. Cases were censored if their sputum cultures never converted before the last follow-up. Also, we used regression for survival analysis, such as Cox Proportional Hazards Regression Analysis with 95% confidence intervals (CIs) to estimate for the effect of each variable on initial culture conversion. Proportional hazards assumptions were assessed by comparing visually estimated −ln(ln) survivor curves, Schoenfeld residual, and supremum test. In the term of missing culture result, we use the midpoint between the first month that sputum culture was not available (NA), and the month that culture showed no growth.

We assessed the sensitivity and specificity of initial culture conversion at the beginning of treatment to 15-month in the prediction of the development of XDR-TB and treatment outcome. We plotted receiver operating characteristic (ROC) curves to see the effect of different time points for sputum culture conversion on balance between sensitivity and specificity. The method we used to determine the cut-off value was the point on the curve with minimum distance from the left-upper corner of the unit square and the point where the Youden's index is maximum (Sensitivity+Specificity -1)(41, 89, 90).

Ethics statement

The Institutional Review Board of the University of California Los Angeles (UCLA IRB#19-000158) and the Ethics Committee for Research in Human Subjects Department of Diseases Control (FWA #00013622) approved this study in 2019. This study was also approved by ten local Institutional review boards from selected hospitals. In addition, 22 hospitals approved the informed consent forms before we conducted the study in their hospitals.

4.4 Results

We researched in order to assess the difference in time to sputum culture conversion among XDR-TB cases while on MDR-TB treatment and MDR-TB control.

4.4.1 Time and predictors of initial sputum culture conversion

Overall, 38 case participants were later diagnosed with XDR-TB, and 76 control participants were not diagnosed with XDR-TB disease. The proportion of sputum culture sent according to guidelines (17) was 21.1 % (23.7 % in MDR-TB cases and 15.8% in the group later developed XDR-TB). However, the proportion of sputum culture sent more than three times in the first six months was 78.1 % (81.6 % in MDR-TB cases and 71.1% in the group later developed XDR-TB). A total of 114 subjects were aged between 18-82 years. Of 38 cases who later developed XDR-TB, 26 (68.4%) had initial sputum culture conversion in a median of 3.5 months (interquartile range [IQR] 2.0-11.5). However, the remaining 12 (10.5%) patients experienced non-conversion. Of 76 cases who did not develop the XDR-TB disease, all had initial sputum culture conversion at 1.5 months (IQR 1.0-2.0). In 102 (89.5%) converters, 78 (68.4%) had sustained conversion, and 24 (21.1%) experienced subsequent positive culture or reversion (figure 4-1).

Univariate Cox Proportional Hazards Regression suggested that improper regimen and nonadherence were associated with less likely culture conversion (table 4-2). In multiple Cox Proportional Hazards Regression, the improper regimen was also found to significantly reduce the chance of culture conversion, controlling for the wrong prescription for dosage and administration, and follow-up sputum culture according to guidelines (17).

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4.4.2 Lack of culture conversion as a prognostic marker for the development of XDR-TB and treatment failure

The maximum combined sensitivity (63.2%) and specificity (94.7%) in predicting the development of XDR-TB were at four-month of MDR-TB treatment (table 4-4). Moreover, the maximum combined of sensitivity (67.7%) and specificity (93.8%) was found at four-month of MDR-TB treatment in order to predict failure outcome (table 4-5).

4.4.3 The pattern of reversion while on MDR-TB treatment

In 102 converters, 24 (20.2%) experienced subsequent positive culture or reversion. The 20 cases did develop to XDR-TB, but four did not. In the group that developed XDR-TB, 13 out of 20 (65%) culture converted before six months, then the culture of ten cases (10/13) turned back to growth in one year. Two out of 13 cases' culture reverted to growth at 13- and 14-month and persisted till the end of MDR-TB treatment. The culture of the last case converted at six-month of treatment and then reverted one month after stopping the MDR-TB treatment.

For four cases who did not develop XDR-TB, the culture converted to no growth before month three of MDR-TB treatment. Three out of four were transiently positive only once and returned to be no growth till cure. The culture of the last case converted to no growth at the beginning of MDR-TB treatment and reverted to growth at month four of treatment; however, the culture converted to no growth again at 18-month and then discharged as a cure after 30-month of treatment.

4.5 Discussion

In this case-control study of 114 participants, 102 (89.5%) patients had sputum culture conversion that was sustained in 78 (68.4%) patients. Overall, 102 cases converted in a median time of two months, which was similar to a study from Holtz, Magee and Yihunie et al. (91-93), but shorter

than reported in the study by Kurbatova, Lu, Velayutham, and Hafkin et al. (86, 87, 94, 95). In the group who developed to XDR-TB disease, the median time of 3.5 months was much shorter than six months in the Lu study for patients with failure or death (87). Shorter median time could be explained by most physicians who might give anti-TB drugs such as SM, INH, RIF, PZA, and EMB while waiting for drug susceptibility test.

Patients receiving an improper regimen were less likely to have culture conversion after controlling for wrong drug administration and dosage and follow-up sputum culture. Yuen et al. also supported that MDR-TB regimens, including more potentially five or more active drugs, were more likely to improve response to MDR-TB treatment (96). In our study, we need only at least four active drugs in the intensive phase, which was less than Yuen's study. However, WHO has launched a new shorter all-oral bedaquiline containing regimen of 9-12 months' duration. The problem of improper regimen would be solved soon.

Based on the interim result, the sputum culture non-conversion at month six of treatment had moderate sensitivity (58.8%) but high specificity (98.8%) for predicting failure results. In addition, using the interim result at six months had moderate sensitivity (52.6%) and the same high specificity (98.7%) for predicting the chance to develop the XDR-TB disease. Nonetheless, by varying the month to report sensitivity and specificity, the sputum non-conversion at four-month gave us the maximum combined sensitivity and specificity for predicting treatment failure (67.7%, 93.8%) and XDR-TB (63.2%, 94.7%).

Our study showed similar results to Javaid's study (97), which recommended using sputum culture conversion at four-month as a surrogate marker in predicting the outcome. However, our result differs from a study by Kurbatova et al. (86), which reported that the maximum combined

sensitivity and specificity in predicting failure was observed at five-month. The benefit of using four-month than five- or six-month is to reduce the time for physicians to evaluate the effectiveness of the current MDR-TB regimen and shorten the time of contagion.

The proportion of non-conversion (12 cases) and patients who had their initial sputum culture conversion after month six of treatment (13 cases) was 25 out of 114 (21.93%). Among 25 cases, 21 cases (84%) developed XDR-TB disease later. The prolonged infectiousness (21.93%) in this group is a source of concern for community transmission (94).

For the cases whose culture converted within six months (89 out of 114 cases or 78.07%), 17 out of 89 cases (19.10%) developed XDR-TB disease later. About three-fourths (13/17) of them reverted to positive. Moreover, one case reverted to growth after stopping MDR-TB treatment, and the growth turned into XDR-TB disease. Therefore, the monthly follow-up of sputum culture and reevaluation by drug susceptibility test when there is no conversion are appropriate to detect the failure and the development to XDR-TB promptly. The extension of follow-up sputum to the post-treatment completion period was also still useful.

This study was subject to several limitations. The most important was missing culture results for some patients, reaching 50% in some months. Then, the absences of the sputum result could bias the results away from the null; however, we reduce this bias by using the midpoint of the first report of missing to first report of no growth. Second, culture and Drug Susceptibility Testing (DST) were performed locally in their region, for which the laboratory techniques could be slightly different between sites. Nonetheless, all 13 regional laboratories participated in quality assurance programs under the supranational TB reference laboratories in Thailand. Third, we chose the participants who were alive and could give us informed-consent in the group who did not develop

the XDR-TB disease. This selection could increase the chance to succeed in this MDR-TB group because of their inherent character to survive, causing survival bias.

In a recent review, there was no similar study in Thailand to predict the development of XDR-TB using the initial culture conversion in their process of treatment of MDR-TB. So, we explore the time to sputum culture conversion could be an excellent prognostic marker for the development of XDR-TB and treatment failure. Our study also showed that an effective regimen and monthly follow-up sputum culture should be provided to ensure the success of MDR-TB treatment. Moreover, sputum culture conversion at four months could be a useful prognostic marker for the development of XDR-TB and the failure outcome.

4.6 Tables and figures

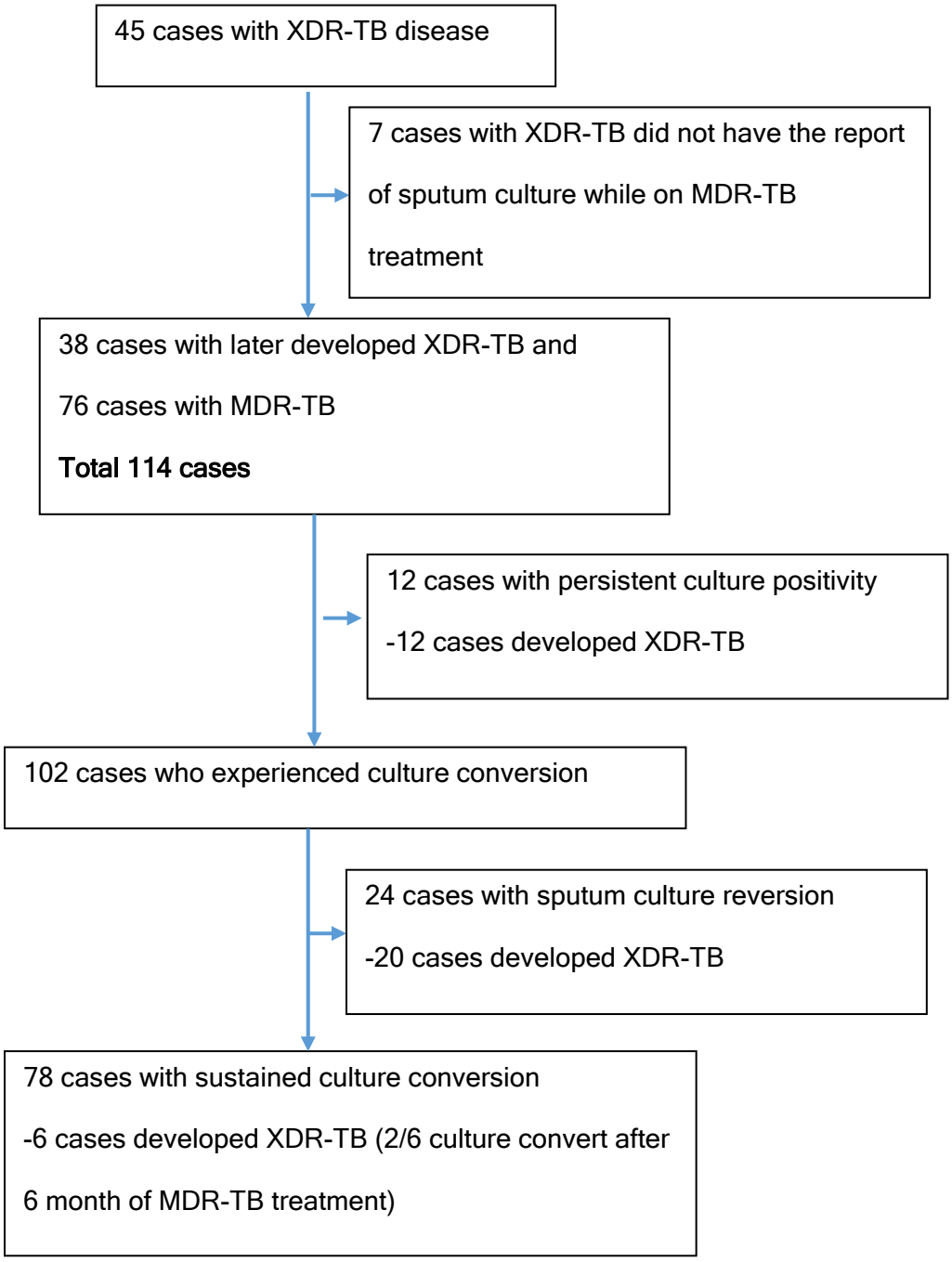


Figure 4-1 Study flowchart (N=114)

Table 4-1 Demographic and clinical characteristics of study participants by drug-resistant pattern (N=114)

			Drug-resistant pattern			
	Overall		MDR-TB		XDR-TB	
	N	(%)	N	(%)	N	(%)
Age group (years)at diagnosis						
15-35	38	(33.3)	22	(28.9)	16	(42.1)
36-55	49	(43.0)	33	(43.4)	16	(42.1)
56-85	27	(23.7)	21	(27.6)	6	(15.8)
Age (median, IQR) at diagnosis	42 (32, 55)		47 (34,59)		40 (27, 52)	
Gender						
Female	34	(29.8)	25	(32.9)	9	(23.7)
Male	80	(70.2)	51	(67.1)	29	(76.3)
BMI Category						
Underweight	54	(47.4)	32	(42.1)	22	(57.9)
Normal	39	(34.2)	29	(38.2)	10	(26.3)
Overweight	10	(8.8)	7	(9.2)	3	(7.9)
Obese	8	(7.0)	6	(7.9)	2	(5.3)
Region						
Central	39	(34.2)	26	(34.2)	13	(34.2)
East	12	(10.5)	8	(10.5)	4	(10.5)
North	12	(10.5)	8	(10.5)	4	(10.5)
Northeastern	21	(18.4)	14	(18.4)	7	(18.4)
South	15	(13.2)	10	(13.2)	5	(13.2)

			Drug-resistant pattern			
	Overall		MDR-TB		XDR-TB	
	N	(%)	N	(%)	N	(%)
West	15	(13.2)	10	(13.2)	5	(13.2)
HIV status						
Negative	109	(95.6)	71	(93.4)	38	(100.0)
Positive	5	(4.4)	5	(6.6)	0	0
Site of TB disease						
Only pulmonary	109	(95.6)	71	(93.4)	38	(100.0)
Pulmonary and extra-pulmonary	5	(4.4)	5	(6.6)	0	0
Cavitary						
No cavity	57	(50.0)	43	(56.6)	14	(36.8)
Cavity disease	57	(50.0)	33	(43.4)	24	(63.2)
Baseline AFB smear status						
Negative	28	(24.6)	23	(30.3)	5	(13.2)
Scanty-1+	26	(22.8)	16	(21.1)	10	(26.3)
2+-3+	44	(38.6)	26	(34.2)	18	(47.4)
Unknown	16	(14.0)	11	(14.5)	5	(13.2)
Previously treated with SLDs						
No	99	(86.8)	74	(97.4)	25	(65.8)
Yes	15	(13.2)	2	(2.6)	13	(34.2)
Treatment delay						
No	100	(87.7)	70	(92.1)	30	(78.9)
Yes	13	(11.4)	6	(7.9)	8	(21.1)

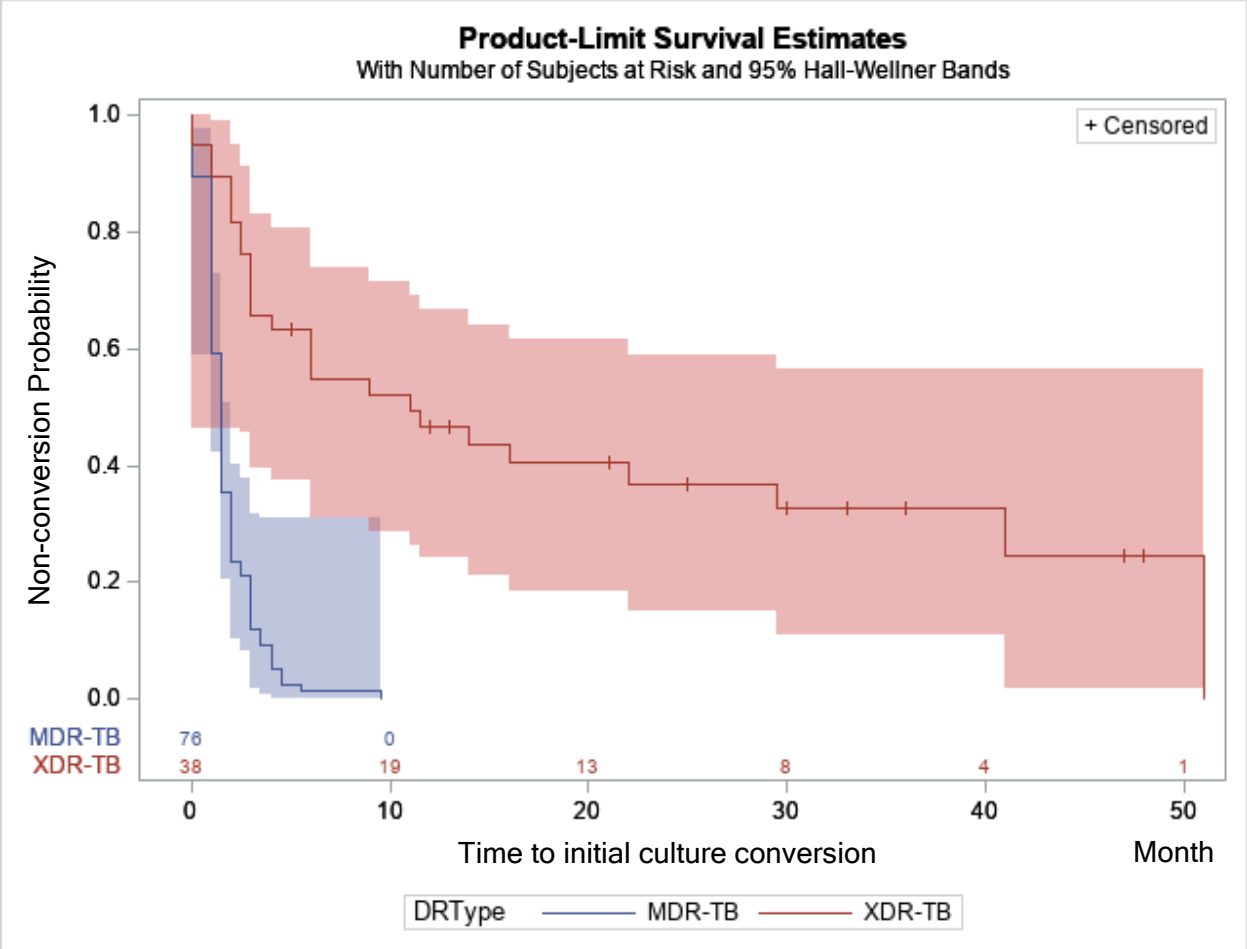
			Drug-resistant pattern			
	Overall		MDR-TB		XDR-TB	
	N	(%)	N	(%)	N	(%)
Follow-up sputum culture according to Guideline						
No	90	(78.9)	58	(76.3)	32	(84.2)
Yes	24	(21.1)	18	(23.7)	6	(15.8)
The outcome of MDR-TB						
Cured	71	(62.3)	67	(88.2)	4	(10.5)
Completed	9	(7.9)	9	(11.8)	0	0
Failed	34	(29.8)	0	0	34	(89.5)

Table 4-2 Univariate analysis of predictors of initial sputum culture conversion among patients with MDR-TB (N=114)

Variable	Number converted/ total cases	Proportion converted (%)	HR	P-value
Gender				
Male	71/80	88.75	0.87 (0.56, 1.33)	0.51
Female	31/34	91.18	1.00	
Age group, years				
15-35	34/38	89.47	1.00	
36-55	43/49	87.75	0.87 (0.55, 1.37)	0.55
56-85	25/27	92.50	1.15 (0.68, 1.94)	0.60
HIV status				
Negative	97/109	88.99	1.00	
Positive	5/5	100.00%	2.43 (0.97, 6.06)	0.06
Smear positivity				
Neg	28/28	100.00%	1	
NA	12/16	75.00%	0.57 (0.29, 1.11)	0.10
Pos	62/70	86.11%	0.69 (0.44, 1.08)	0.10
Diabetes Mellitus				
No	72/80	90.00%	1	
Yes	30/34	88.24%	0.78 (0.50, 1.20)	0.25
Non-adherence				
No	88/95	92.63%	1.00	
Yes	14/19	73.68%	0.58 (0.33, 1.021)	0.06
Improper regimen				
No	63/63	100.00%	1.00	
Yes	39/51	76.47 %	0.26 (0.16, 0.42)	<.0001
Wrong drug administration and dosage				
No	14/14	100.00%	1.00	
Yes	18/100	18%	0.63 (0.36, 1.12)	0.11
Follow-up sputum culture according to guideline				
No	79/90	87.78%	1.00	
Yes	23/24	95.83%	1.57 (0.97, 2.51)	0.06

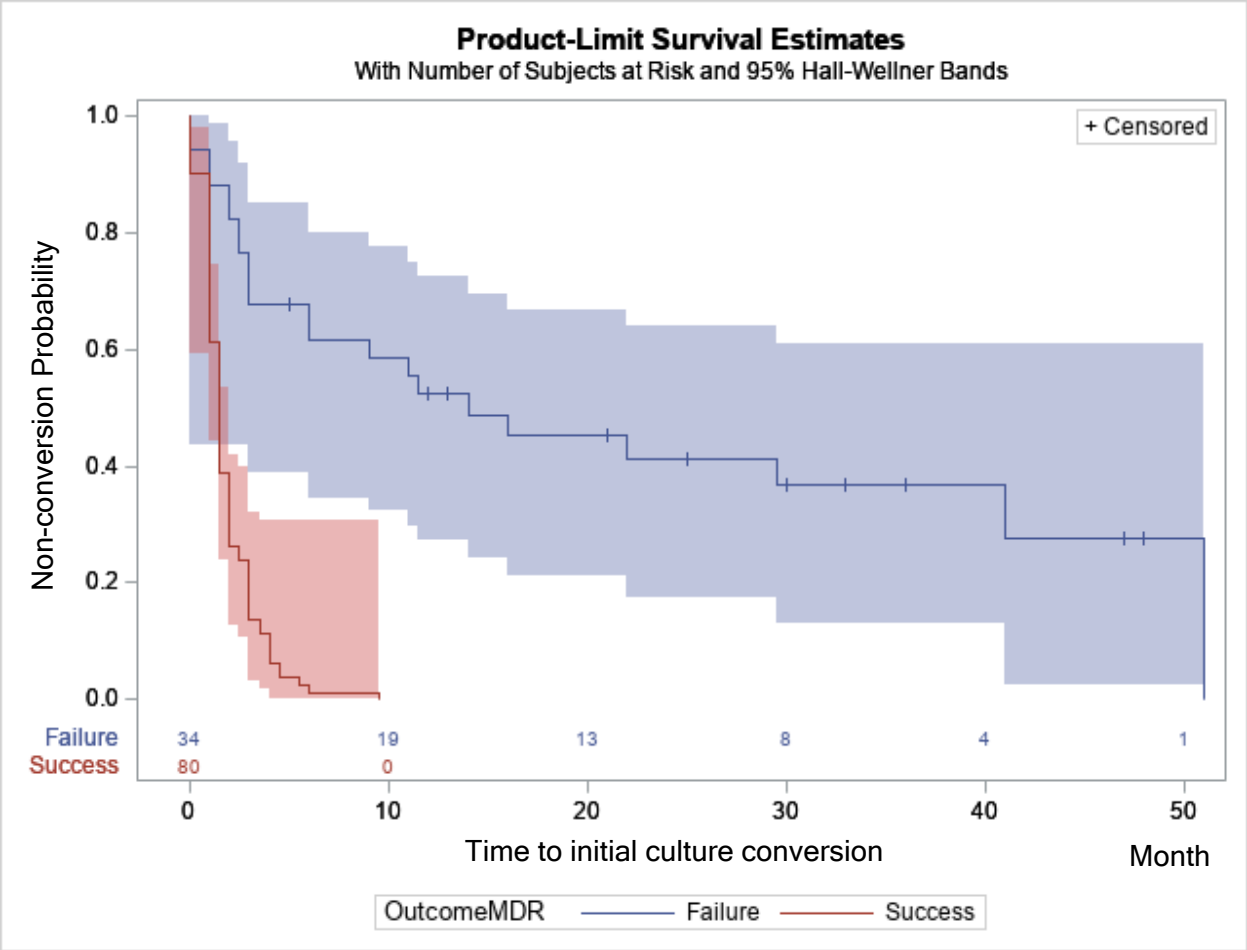
Table 4-3 Multivariable analysis of predictors of initial sputum culture conversion among patients while treated with MDR-TB (N=114)

Variables	aHR (95%CL)	P-value
Improper regimen *		
No	1.00	
Yes	0.24 (0.15, 0.38)	<.0001
Wrong drug administration and dosage		
No	1.00	
Yes	0.78 (0.42, 1.44)	0.4268
Follow -up sputum culture		
No	1.00	
Yes	1.22 (0.73, 2.04)	0.4542



P value <.0001
Crosses depict censored patients.

Figure 4-2 Time to sputum culture conversion in patients with MDR-TB tuberculosis, by drug-resistant pattern (Developed XDR-TB vs. Not developed XDR-TB, N=114)



P value <.0001
Crosses depict censored patients.

Figure 4-3 Time to sputum culture conversion in patients with MDR-TB tuberculosis, by treatment outcome (failure vs. success, N=114)

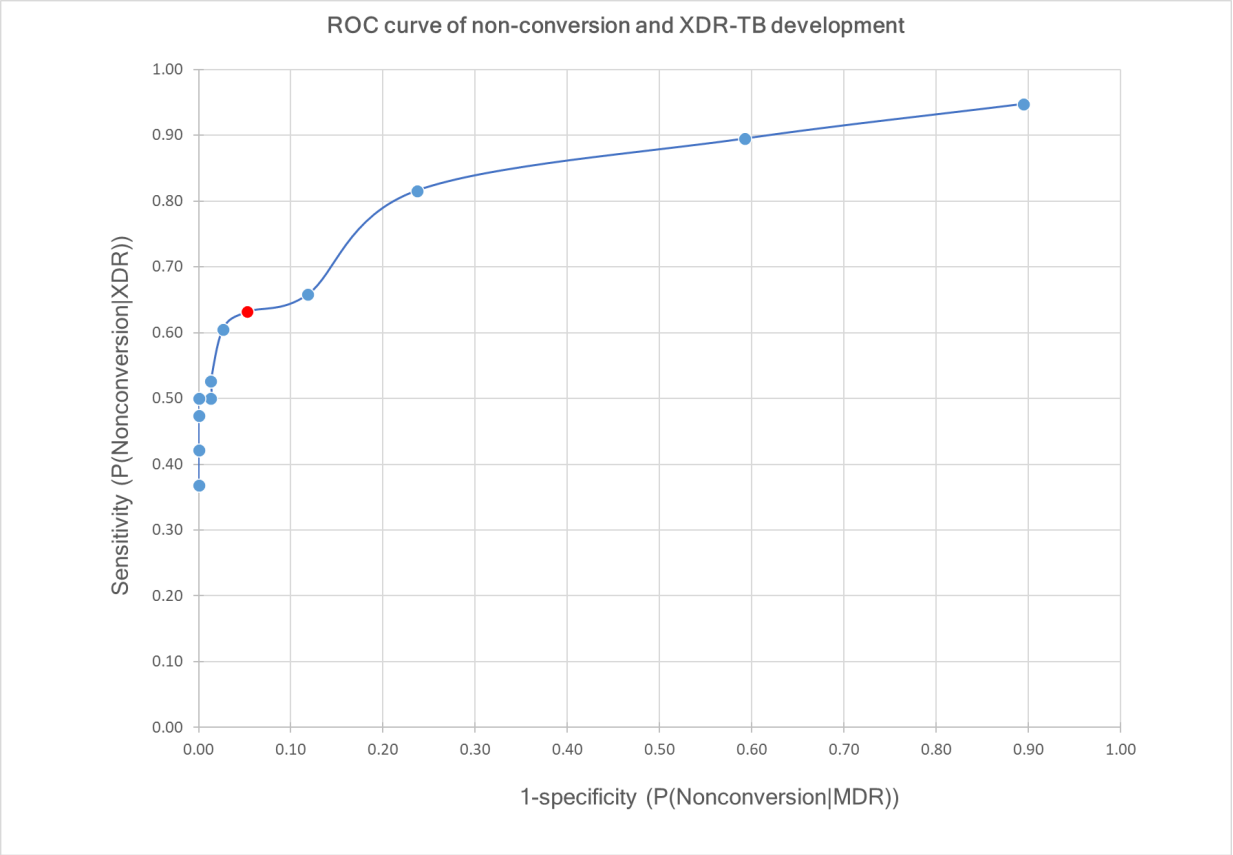


Figure 4-4 Receiver operating characteristic curve for predicting the development of XDR-TB based on time to sputum culture conversion

Table 4-4 Sensitivity, specificity of different time points of interim outcome assessment of initial culture conversion in predicting the development of XDR-TB

Label: non-conversion	M->XDR	MDR	Sensitivity	Specificity
At the beginning	36	68	94.74%	10.53%
1-Month	34	45	89.47%	40.79%
2-Month	31	18	81.58%	76.32%
3-Month	25	9	65.79%	88.16%
4-Month	**24**	**4**	**63.16%**	94.74%
5-Month	23	2	60.53%	97.37%
6-Month	20	1	52.63%	98.68%
7-Month	20	1	52.63%	98.68%
8-month	20	1	52.63%	98.68%
9-month	19	1	50.00%	98.68%
10-month	19	0	50.00%	100.00%
11-month	18	0	47.37%	100.00%
12-month	16	0	42.11%	100.00%
15-month	14	0	36.84%	100.00%

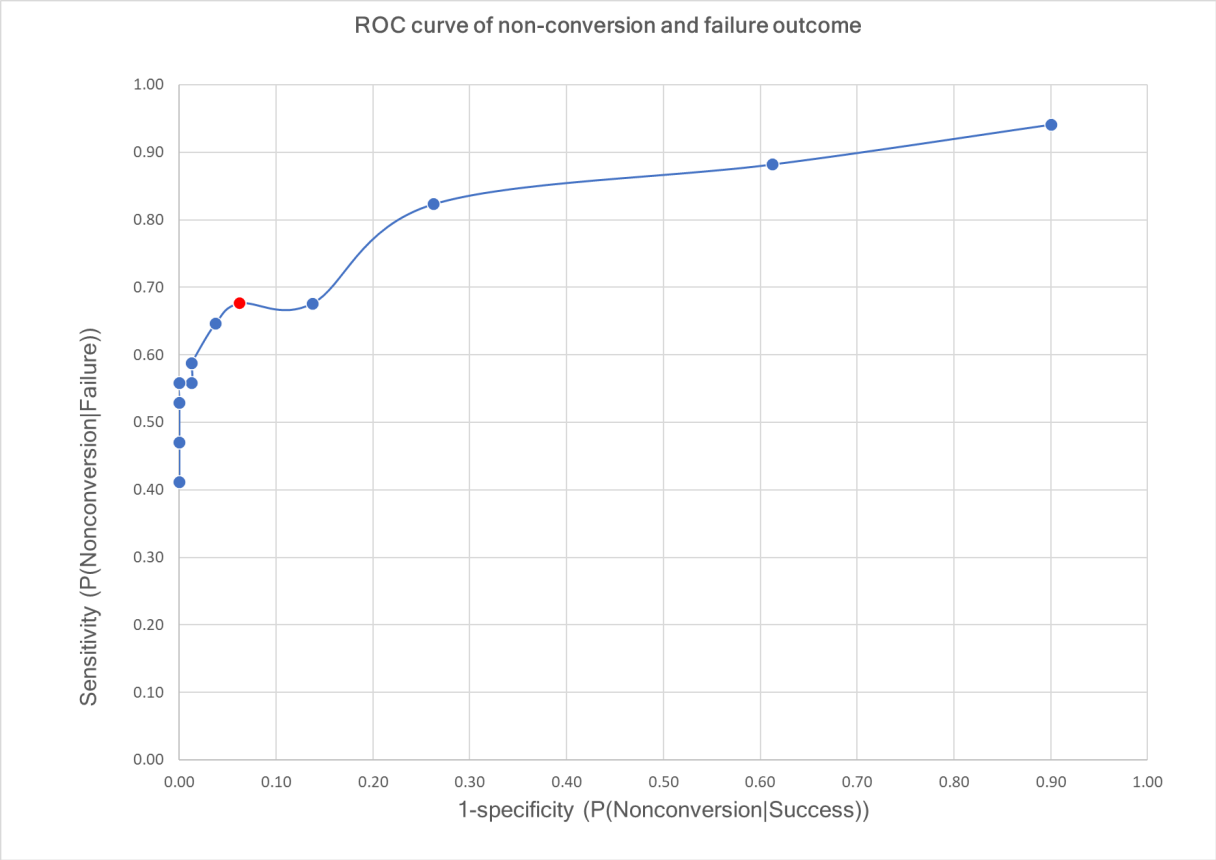


Figure 4-5 Receiver operating characteristic curve for predicting the failure outcome based on time to sputum culture conversion

Table 4-5 Sensitivity, specificity of different time points of interim outcome assessment of initial culture conversion in predicting the failure outcome vs. success

Label: non-conversion	Failure	Success	Sensitivity	Specificity
At the beginning	32	72	94.12%	10.00%
1-Month	30	49	88.24%	38.75%
2-Month	28	21	82.35%	73.75%
3-Month	23	11	67.65%	86.25%
4-Month	**23**	**5**	**67.65%**	**93.75%**
5-Month	22	3	64.71%	96.25%
6-Month	20	1	58.82%	98.75%
7-Month	20	1	58.82%	98.75%
8-month	20	1	58.82%	98.75%
9-month	19	1	55.88%	98.75%
10-month	19	0	55.88%	100.00%
11-month	18	0	52.94%	100.00%
12-month	16	0	47.06%	100.00%
15-month	14	0	41.18%	100.00%

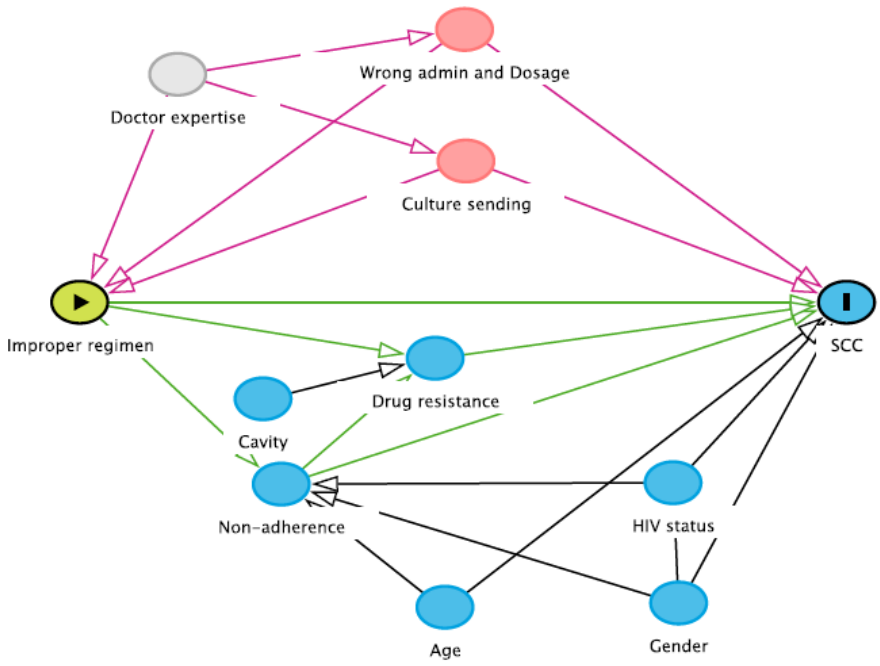


Figure 4-6 Directed Acyclic Graph for Time to sputum culture conversion

Chapter 5 Conclusion and public health implications

Extensively drug-resistant tuberculosis (XDR-TB) is the most dangerous drug-resistant TB with low treatment success rate, more toxic adverse effects, and the highest costing treatment regimen. So, it is crucial to explore the risk factors for XDR-TB. Many studies have studied the risk factors for XDR-TB development, either clinical, programmatic part, or psychological part; nonetheless, few studies explored all these aspects together. These studies examined all essential components together in order to identify the risk for development to XDR-TB comprehensively. These findings could be used for a policy framework to increase the effectiveness of the drug-resistant tuberculosis course of treatment.

Our first study (Chapter 2) demonstrated that improper regimen and insufficient duration of treatment are the main factors in XDR-TB development, followed by non-adherence (missing dose more than 10 % of the total intended course). Family emotional support is also found to be a preventive factor. Therefore, we must better focus on treatment audits and strengthening the consultation process of MDR-TB treatment to improve the quality of treatment. Moreover, emotional support, especially from family, and understanding of the importance of treatment adherence, will help protect patients from experiencing further drug-resistance.

Our second study (Chapter 3) revealed that *NAT2 *4/5B* was believed to be associated with XDR-TB. However, no association was seen about the acetylation type and XDR-TB. In the part of HLA class2 (DRB1, DQB1), only the heterozygosity of *HLA -DRB1*16:02,* which related to adult-onset immunodeficiency, was seen more in cases with XDR-TB. Future research should focus on *HLA-DRB1*16:02*, and autoantibody to IFNγ in a larger group.

Our third study (Chapter 4) showed a significant pattern in sputum culture conversion. We found that the group who later developed XDR-TB had a long time to conversion, which was 3.5 months compared with 1.5 months in the group who did not develop it. Again, the improper regimen and insufficient duration of treatment played an essential role in culture conversion. When we considered the cut-off point of culture conversion for determining the risk of XDR-TB development, the lack of culture conversion at four months of treatment gave us a maximum combination of high sensitivity (63.2%) and specificity (94.7%). For the cases which already culture converted, most of them who developed XDR-TB had reverted their culture in one year. Therefore, a monthly follow-up sputum culture after the intensive phase and the reevaluation by drug susceptibility test when persistent culture growth was necessary to detect treatment failure and XDR-TB.

In summary, the findings in this dissertation suggest possible risk and protective factors for the development of XDR-TB. Previous studies in Thailand have found a higher proportion of poor outcomes of MDR-TB treatment, and severe complication was detected when patients were diagnosed with XDR-TB. Since more cases with XDR-TB are a significant concern right now at the country, regional, and global levels. Our findings suggest possible ways to reduce the progression of cases with XDR-TB.

From a policy perspective, future studies are needed to set up the consultation panels and audit system and evaluate the impact after implementation. Further work may also be needed to focus the host genetic such as *HLA-DRB1*16:02* and autoantibody to IFNγ in a larger group. Next, whole-genome sequencing of TB organisms has to be done in order to know that the XDR-TB came from either relapse with the same strain while they were infected with MDR-TB, or reinfection with the community transmission of XDR-TB strain. This difference could lead to specific measures to prevent XDR-TB development. Lastly, the monthly sputum follow-up and

reevaluation by drug susceptibility test should be encouraged for early detection of failure and the start of effective treatment immediately.

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