Occurrence of multidrug-resistant *Mycobacterium tuberculosis* in upper Southern Thailand

อุบัติการณ์เชื้อ *Mycobacterium tuberculosis* ที่ดื้อยาหลายขนานในพื้นที่ภาคใต้ตอนบนของประเทศไทย

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กรมควบคุมโรค กระทรวงสาธารณสุข

Occurrence of multidrug-resistant *Mycobacterium tuberculosis* in upper Southern Thailand

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Preface

The objective of this research report is to investigate the occurrence and characteristics of drug resistance in *M. tuberculosis* among patients in upper Southern Thailand. Multidrug-resistant tuberculosis (MDR-TB-TB) and extensively drug-resistant tuberculosis (XDR-TB-TB) are additional challenges due to their resistance to multiple first-line drugs, and *Mycobacterium tuberculosis* raises global concerns. This study examined the occurrence of TB, antibiotic resistance as a result of *inhA* and *katG* gene mutations, and multidrug resistance in *M. tuberculosis* during the fiscal years 2020–2022.

Thank you to everyone who participated in the research study. Until it was successfully completed.

Pathom Karaipoom

บทคัดย่อ

วัณโรคที่เกิดจากเชื้อ *Mycobacterium tuberculosis* ยังคงเป็นปัญหาด้านสุขภาพที่สำคัญทั่วโลก การศึกษานี้มีวัตถุประสงค์เพื่อตรวจสอบอุบัติการณ์ของวัณโรคและการดื้อยาปฏิชีวนะแบบย้อนหลังที่เกี่ยวข้องกับการกลายพันธุ์ของยีน *inhA* และ *katG* ใน *M. tuberculosis* ในช่วงปีงบประมาณ 2563-2565 เก็บตัวอย่างจากผู้ป่วยในโรงพยาบาลที่ตั้งอยู่ใน 7 จังหวัดภาคใต้ตอนบนของประเทศไทย ศึกษาความไวต่อยาต้านจุลชีพและการตรวจหาการดื้อยา isoniazid ที่เกี่ยวข้องกับการกลายพันธุ์ของยีน *inhA* และ *katG* ใน *M. tuberculosis* สิ่งส่งตรวจจำนวน 19,186 ตัวอย่าง ถูกส่งมาตรวจวินิจฉัยทางห้องปฏิบัติการของสำนักงานป้องกันควบคุมโรคที่ 11 จังหวัดนครศรีธรรมราช โดยกลุ่มตัวอย่างร้อยละ 51 ได้รับจากผู้ป่วยในจังหวัดนครศรีธรรมราช รองลงมาคือจังหวัดสุราษฎร์ธานี สำหรับการกระจายเชิงพื้นที่ของผู้ป่วยที่ติดเชื้อวัณโรคอุบัติการณ์ของวัณโรคอยู่ในระดับสูงในจังหวัดที่มีความหนาแน่นของประชากรปานกลางถึงสูง อัตราการเกิดวัณโรคโดยเฉลี่ยสูงสุดในการศึกษานี้พบในจังหวัดภูเก็ต 9.75 รายต่อประชากรแสนคน การดื้อยา isoniazid ที่ตรวจพบคือ 394, 255 และ 179 ราย ในปี พ.ศ. 2563, 2564 , 2565 ตามลำดับ และพบการดื้อยาชนิด MDR-TB, pre-XDR-TB และ XDR-TB จำนวนรวมทั้งหมดคือ 99, 12 และ 4 ราย ตามลำดับ การดื้อต่อยา isoniazid ที่เกี่ยวข้องกับการกลายพันธุ์ของ *inhA* คือ 192, 142 และ 105 ราย ในขณะที่การดื้อต่อยา isoniazid ที่เกี่ยวข้องกับการกลายพันธุ์ของ *katG* คือ 249, 182, 120 ราย ในปี พ.ศ. 2563, 2564 และ 2565 ตามลำดับ การศึกษานี้แสดงให้เห็นว่าการทดสอบการดื้อยา rifampicin เพียงอย่างเดียวไม่เพียงพอ เนื่องจากมีการดื้อต่อยา isoniazid สูง ควรพิจารณาเพิ่มวิธีการตรวจการดื้อต่อยา isoniazid ทั้ง genotypic และ phenotypic ของผู้ป่วยวัณโรคทุกรายในพื้นที่ภาคใต้ตอนบนของประเทศไทย รวมถึงการศึกษาเพิ่มเติมของตำแหน่งกลายพันธุ์ในยีน *inhA* และ *katG* ซึ่งส่งผลต่อการดื้อยา isoniazid ที่ระดับแตกต่างกัน เป็นการเพิ่มประสิทธิภาพในการรักษา ป้องกันและควบคุมการแพร่กระจายของวัณโรคดื้อยา

คำสำคัญ: เชื้อมัยโคแบคทีเรียม, ไอโซไนอะซิด, ยีน *inhA* และ *katG*, การดื้อยาหลายขนาน, ภาคใต้ตอนบนของประเทศไทย

Abstract

Tuberculosis, caused by *Mycobacterium tuberculosis,* remains a significant global health concern. This study aimed to retrospectively investigate the prevalence of tuberculosis and antibiotic resistance associated with a mutation in *the inhA* and *katG* genes in *M. tuberculosis* during the fiscal years 2020–2022. The samples were collected from the patients in the hospitals located in seven provinces in upper Southern Thailand. The antimicrobial susceptibility and detection of isoniazid resistance associated with *inhA* and *katG* gene mutations in *M. tuberculosis* were investigated. A total of 19,186 samples were sent to the Office of Disease Prevention and Control Region 11st, Nakhon Si Thammarat, Thailand. In terms of the spatial distribution of TB-infected cases, the incidence of TB was high in the province, which has a moderate to high population density. Fifty-one percent of the samples were received from patients located in Nakhon Si Thammarat, followed by Surat Thani provinces. For the spatial distribution of TB-infected cases, the incidence of TB was high in the province, which has a moderate to high density of population. The highest average incidence of TB in this study was found in Phuket province (9.75 per 100,000 risk person-years). The detected isoniazid resistance was 394, 255, and 179 cases in the years 2020, 2021, and 2022, respectively. A total of 99, 12, and 4 isolates were classified as MDR-TB, pre-XDR-TB, and XDR-TB, respectively. The antimicrobial resistance associated with *the inhA* mutation was 192, 142, and 105 isolates, while the resistance associated with *the katG* mutation was 249, 182, and 120 cases in 2020, 2021, and 2022, respectively. This study shows that testing for rifampicin resistance alone is insufficient due to the high prevalence of isoniazid resistance. It is recommended to incorporate both genotypic and phenotypic testing for isoniazid resistance in all tuberculosis patients in the upper southern region of Thailand. Additionally, to enhance treatment efficiency and to prevent and control the spread of drug-resistant tuberculosis, further studies on the mutation sites in the *inhA* and *katG* genes that affect isoniazid resistance at different levels are necessary.

Keywords: *Mycobacterium tuberculosis*, isoniazid, *inhA* and *katG* genes, multi-drug resistance, upper Southern of Thailand

Acknowledgments

This work was funded by the Center of Excellence in Innovation of Essential Oil and Bioactive Compounds (Grant no. WU-COE-65-05), Walailak University, Thailand. The authors thank the staff at the Office of Disease Prevention and Control region 11st, Nakhon Si Thammarat, Thailand, for laboratory services and sample collection. We would like to thank One Health Research Center, Walailak University, Thailand, for their support.

Pathom Karaipoom

Contents

List of Table

List of Figure

List of Abbreviation

AFB	acid- fast bacilli
AG	aminoglycosides
DM	Diabetes Mellitus
DOT	directly observed treatment
DR-TB	drug resistant tuberculosis
FQs	fluoroquinolones
Hr-TB	isoniazid resistant tuberculosis
H	isoniazid
IC	infection control
IGRAs	Interferon-gamma release assay
LPA	line probe assay
LTBI	latent tuberculosis infection
MAC	Mycobacterium avium complex
MDR-TB	multidrug resistant tuberculosis
MTBC	Mycobacterium tuberculosis complex
NAT2	arylamine N-acetyltransferase type 2
NTIP	National Tuberculosis Information Program
NTM	nontuberculous mycobacterium
R, RIF	rifampicin
RR	rifampicin resistant
RR-TB	rifampicin resistant tuberculosis
S	streptomycin
SLD	second line drugs
TPT	tuberculosis preventive treatment
TST	tuberculin skin test
WHO	World Health Organization
XDR-TB	extensively drug resistant tuberculosis
Z	pyrazinamide

Chapter 1

Introduction

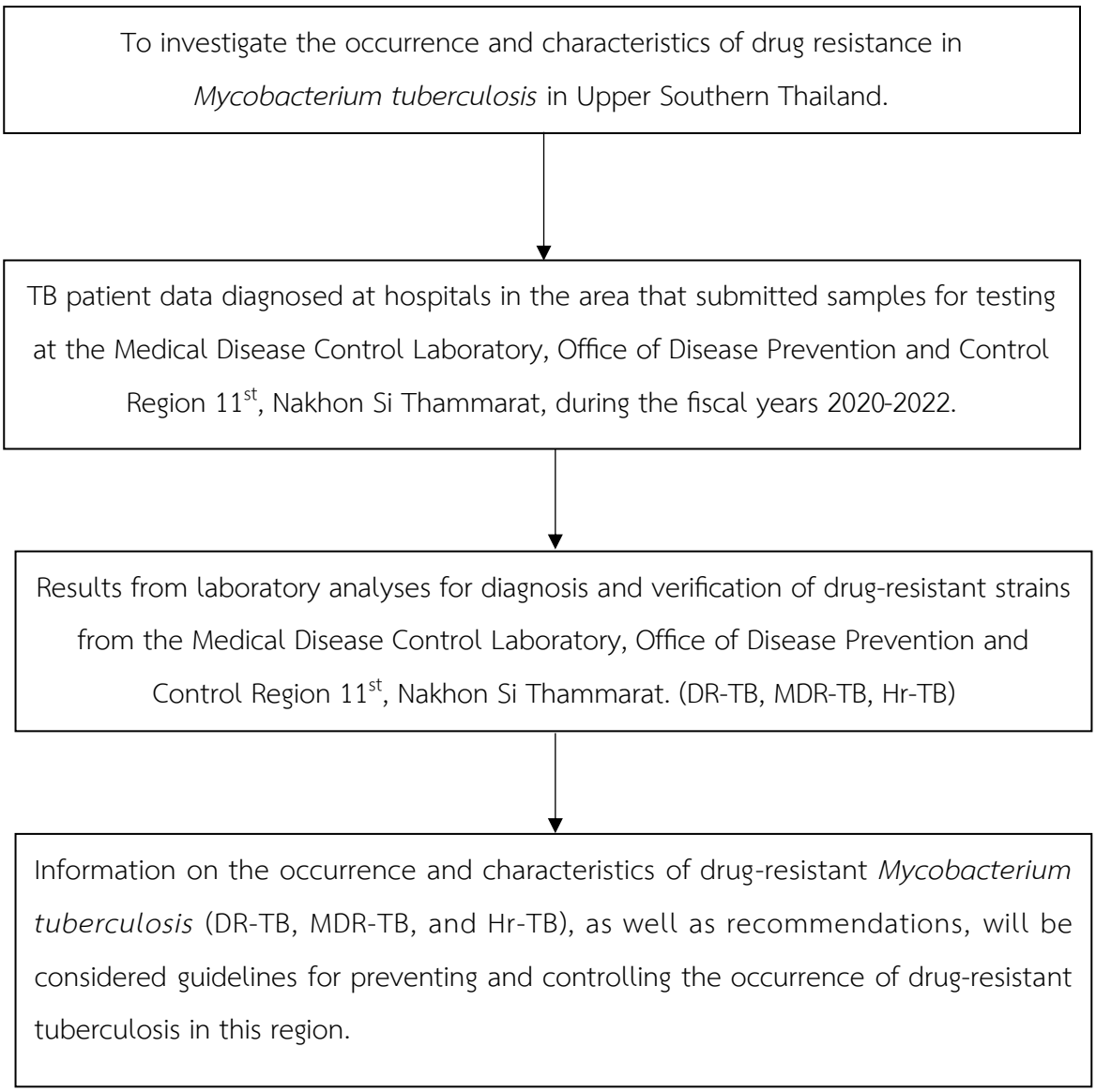
Mycobacterium tuberculosis infections continue to pose a major global health issue, marked by high morbidity and mortality rates. 10.6 million new tuberculosis (TB) cases were reported by the World Health Organization (WHO) in 2021 [1]. In many organs, including the lung, lymph nodes, and bone, TB is primarily caused by the pathogen. *M. tuberculosis* infections result in diverse clinical manifestations, ranging from inapparent to active pulmonary or extrapulmonary (EP) disease. Pulmonary TB is the most common clinical form of the illness and is responsible for the majority of TB cases that have been documented globally [2]. EP-TB, a form of *M. tuberculosis* infection outside the lungs, is a significant cause of morbidity and mortality in humans [2].

Multidrug-resistant TB (MDR-TB) results from *M. tuberculosis* strains that resist treatment with isoniazid and rifampicin, two primary anti-TB medications [3]. TB infection is treated with first-line drugs isoniazid and rifampicin. The combination of isoniazid, rifampicin, and pyrazinamide effectively cures *M. tuberculosis* strains for 3 months that are fully drug-susceptible [4]. Failure to comply with treatment or take adequate medication contributes to the development and spread of drug resistance in *M. tuberculosis* against all relevant antibiotics [5]. MDR-TB is TB caused by *M. tuberculosis* that is resistant to treatment with at least two of the first-line anti-TB drugs, including isoniazid and rifampicin [3]. Furthermore, extensively drug-resistant TB (XDR-TB) is defined as MDR-TB plus resistance to one of the fluoroquinolones and to one of the second-line injectable anti-TB drugs: Amikacin, kanamycin, or capreomycin [6].

Isoniazid is a commonly used drug for treating *M. tuberculosis* infections. Inhibiting NADH-dependent enoyl-ACP reductase, encoded by *inhA*, prevents mycolic acid synthesis [7]. Isoniazid acts as a prodrug that requires activation by the *M. tuberculosis* catalase-peroxidase enzyme *katG*. Mutations in *katG* and *inhA* genes confer resistance to isoniazid [7]. Infection control is becoming more challenging because of rising drug resistance, especially to the first-line antibiotic isoniazid, and the occurrence of MDR-TB [8].

This study aimed to investigate the occurrence and characteristics of drug resistance in *M. tuberculosis* among patients in upper Southern Thailand during the fiscal year 2020-2022. The seven provinces in this area are Chumphon, Surat Thani, Nakhon Si Thammarat, Phuket, Krabi, Phang Nga, and Ranong, as outlined in the following research framework.

Research Framework



Chapter 2

Literature Reviews

2.1Tuberculosis

Tuberculosis also known as TB is a bacterial infection caused by *Mycobacterium tuberculosis*. It is well-known that TB often affects the lungs but it can also affect other parts of the body including lymph node and bone marrow. Droplets carrying the tuberculosis germs are discharged into the air and dispersed across the community when an individual with the disease coughs or sneezes. World Health Organization (WHO) has reported that a quarter of the world's population has infected with *M. tuberculosis*. Interestingly, 5-10% of those who have TB infection will have symptoms and develop TB illness [9]. Tuberculosis continues to be a major global health concern, especially in underdeveloped nations with restricted access to medical care. It is noticed that the risk of contracting tuberculosis is higher in individuals with HIV/AIDS and other immune system disorders than in healthy individuals [10].

Tuberculosis can present with a variety of symptoms, which can vary depending on whether the infection is active (infectious) or latent (dormant). Active TB is simple to transmit to other people. Many common symptoms of active tuberculosis include persistent cough, chest pain, weight loss, fatigue, low-grade fever, night sweats, loss of appetite, joint pain, and swelling of the lymph nodes. Tuberculosis can occur in every organ of the body, most often occurring in the lungs (80 percent) [11], which can spread easily. It can also exist in organs beyond the lungs, such as the lungs and lymph nodes. These organs include the spine, joints, abdomen, urinary system, and nervous system, among others.

There are three categories into which mycobacterium bacteria fall:

1. *Mycobacterium tuberculosis* complex (MTBC) is the cause of tuberculosis in humans and animals. There are eight species in this group, with *Mycobacterium africanum* being the most common. Other *Mycobacterium tuberculosis* species commonly found in this group, such as *Mycobacterium africanum*, are found in Africa. *Mycobacterium bovis* often causes disease in animals, which may contact people by consuming unpasteurized milk. It is the strain used to make the BCG vaccine.

2. non-tuberculous mycobacteria (NTM) comprises over 140 species, including *Mycobacterium avium* complex (MAC), which can be found in the environment, soil, water, and in animals like birds. Most do not cause disease in humans, except in people with weakened immune systems.

3. *Mycobacterium laprae* is the cause of leprosy.

Mycobacterium bacteria is rod-shaped, approximately 0.3 micrometers thick, and approximately 2–5 micrometers long. The stain with Ziehl-Neelsen is red. Tuberculosis does not have a capsule. It does not produce spores that are unable to move. Growth is dependent on oxygen. Aerosols contain tuberculosis bacteria. When a patient coughs or sneezes, it can float in the air for up to 30 minutes. *Mycobacterium tuberculosis* is highly durable in the environment. Many factors, including certain chemicals, heat, sunlight, and ultraviolet light, can destroy it. Sunlight can destroy TB germs in sputum in 20–30 hours, and if exposed to ultraviolet rays, they will die within 1-2 minutes. If not exposed to sunlight, tuberculosis bacteria in dry sputum can survive for up to 6 months. Temperatures of 60 degrees Celsius for 20 minutes can destroy tuberculosis bacteria. [11]

People contract tuberculosis through airborne transmission. Patients with tuberculosis spread out droplets (droplet nuclei) when they cough, sneeze, speak loudly, shout, laugh, or sing. Very large droplets fall to the ground and dry up. Droplets as small as 1–5 micrometers will float and spread in the air. When individuals inhale aerosols containing TB germs, the large particles lodge in their noses or throats. This usually does not cause disease, but small particles can enter the air sacs in the lungs.

The factors that affect the spread of tuberculosis infection can be divided into three areas.

1.Factors associated with tuberculosis patients include the presence of pulmonary tuberculosis. windpipe or larynx When bacteria are present in the sputum, Patients with pulmonary ulcers have many infections. If you have a cough, sneeze, or other symptoms that cause heavy breathing,

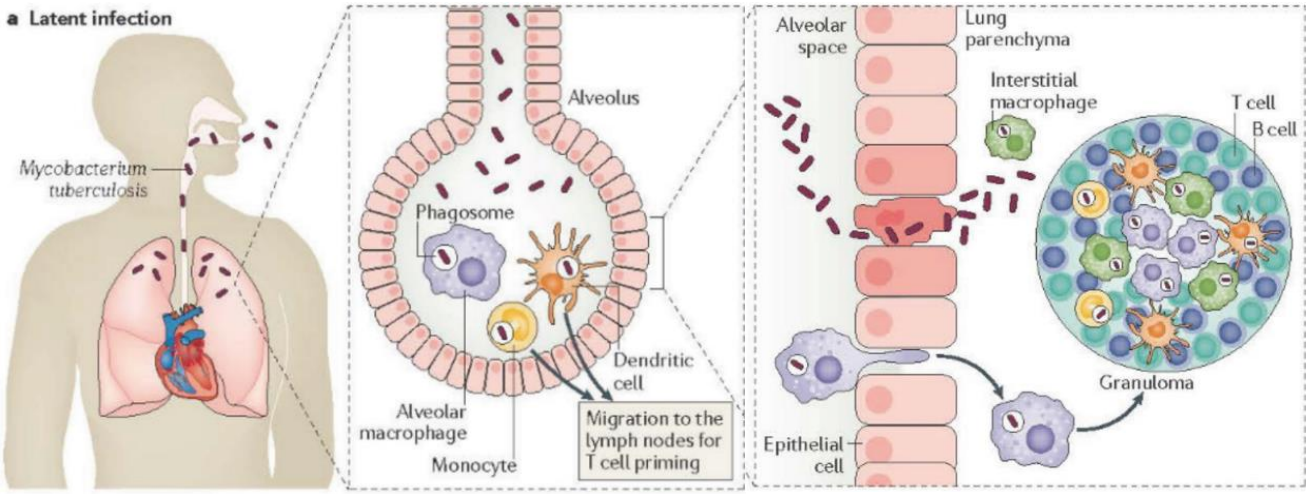
2.Environmental factors include places that are dense and cramped. The sunlight doesn't reach. poor ventilation

3.Service system factors include delays in diagnosis and treatment. Incorrect medication administration; incomplete treatment Engaging in actions that generate aerosols, like inducing coughing, among others

Breathing in germs causes both infection and TB disease. TB enters the air sacs of the lungs. Macrophages in the air sacs are the first immune system that can eliminate the infection, preventing TB infection.

If the first line of immunity is unable to completely eliminate the infection, Tuberculosis bacteria are dividing and increasing in number. The bacteria can penetrate the alveolar walls, where the virus can enter directly, or they can enter the lung tissue through macrophages infected with *M. tuberculosis*. At the same time, dendritic cells and monocytes

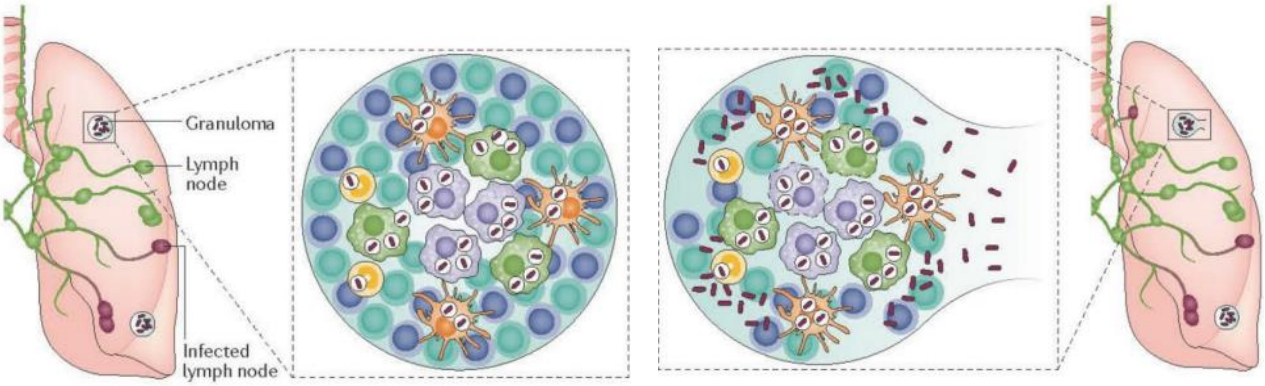
carry *M. tuberculosis* germs to the lymph nodes in the lungs to create T cells, causing an immune stimulation process. Both T cells and B cells gather in the lung tissue. It surrounds a group of macrophages and other cells that contain TB bacteria, known as a granuloma. It is a latent TB infection process.[11] (Figure-1)



Source: Pai M, *et al*. Tuberculosis. Nat Rev Dis Primers. 2016

Figure-1: The pathogenesis of latent tuberculosis infection, also known as LTBI.

If the TB bacteria in the granuloma keep dividing and multiplying and become too large for the granuloma to control, they can enter the bloodstream, spread to various organs, or return to the respiratory system. It can cause pathology in the lungs and/or other organs. This is the active phase of tuberculosis disease. [11] (Figure-2)



Source: Pai M, *et al*. Tuberculosis. Nat Rev Dis Primers. 2016.

Figure-2: Shows the pathogenesis of active TB disease.

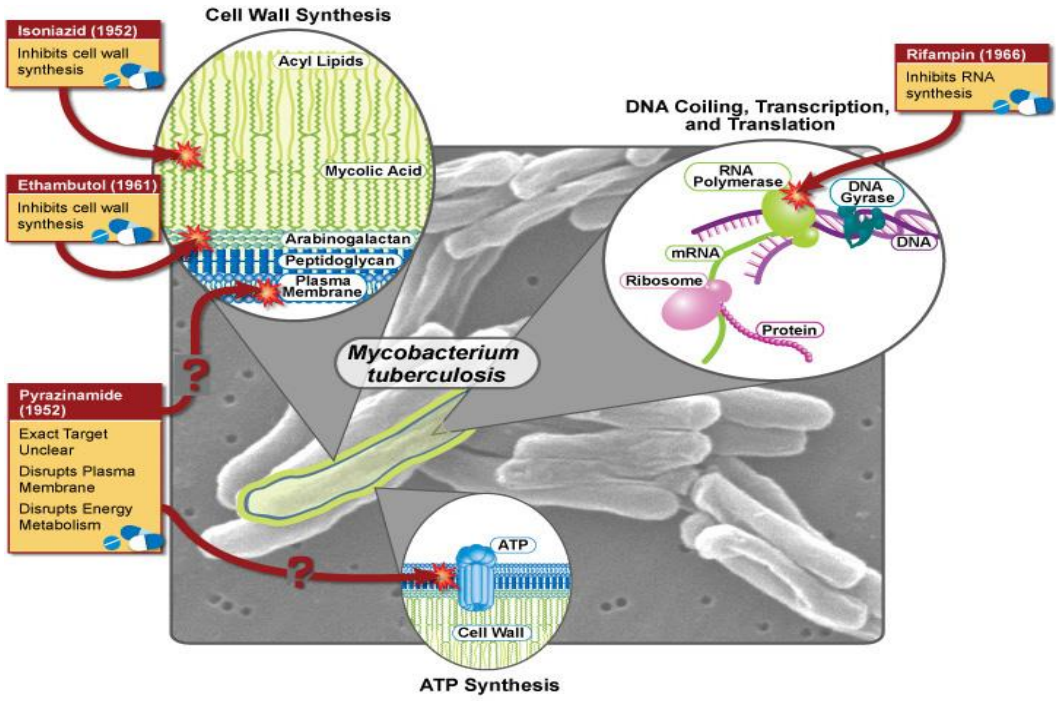
TB treatment typically involves the administration of antibiotics. The treatment typically uses a combination of antibiotics to prevent antibiotic resistance. The most common antibiotics used are isoniazid, rifampicin, pyrazinamide, and ethambutol. Treatment plans might differ based on a number of variables, including the patient's medical history, the kind of TB (drug-sensitive or drug-resistant), and local resistance trends. The TB treatment contains two phases, including the initial phase and the continuation phase. The initial phase typically lasts for 2 months and involves taking a combination of antibiotics to rapidly kill *M. tuberculosis*. The continuation phase is the continuation of treatment after the initial phase. It usually takes 4 to 6 months. The treatment regimen may simplify during this phase, often concentrating on isoniazid and rifampicin. Each type has different effects. (Figure-3).

1.Isoniazid (H): inhibits the synthesis of mycolic acid, which is an important component of the cell wall of mycobacteria. To be effective, it needs enzymes. The mycobacterial catalase peroxidase (*Kat G*) of the infection has an active form. The active form of INH binds with Acyl carriers' protein (AcpM) and beta-ketoacyl carrier protein synthase (KasA), which are important components in the synthesis of mycolic acid. Binding covalently inhibits mycolic acid synthesis, causing the drug to inhibit bacterial cell wall formation.

2. Rifampicin (R): binds to the ß-subunit of bacterial DNA-dependent RNA polymerase and inhibits infection RNA synthesis. Rifampin only binds to bacterial RNA polymerase, not RNA. It inhibits the production of the infection's DNA by binding to the polymerase of eukaryotic cells.

3. Pyrazinamide (Z): inhibits the enzyme fatty acid synthase, which is an enzyme that allows bacteria to synthesize fatty acids for themselves. Additionally, the drug binds to the ribosomal protein S1, thereby inhibiting the process. The aforementioned mechanism triggers trans-translation, the process in bacteria that produces proteins. As a result, the infection is unable to grow.

4. Ethambutol (E): is an inhibitor of the enzyme mycobacterial arabinosyl transferase, which is an enzyme in the process of polymerization of arabinoglycan, which is an important component of the mycobacterial cell wall, thus causing the drug to inhibit the creation of the cell wall of the bacteria.



Source: The Centers for Disease Control and Prevention, CDC/ Dr. Ray Butler; Janice Carr

Figure-3: Shows the effectiveness of drugs to treat tuberculosis.

2.2 *Mycobacterium tuberculosis*

Mycobacterium tuberculosis (*M. tuberculosis*) also known as Koch's bacillus, is a species of pathogenic bacteria in the family *Mycobacteriaceae*. The bacterium is well-known as a species of pathogenic bacteria that causes TB in humans. The bacterium is an acid-fast, non-motile, rod-shaped cell that mostly affects the lungs but can potentially spread to other body areas. Composition of *M. tuberculosis* cell wall contains a structure rich in lipids such as mycolic acids, arabinogalactan, peptidoglycan, and lipoarabinomannan. Mycolic acid is long-chain fatty acids that contains up to 90 carbons and are covalently bonded to the cell wall's arabinogalactan layer. The presence of mycolic acid makes the bacterium resistant to staining by conventional dyes (acid-fast). Furthermore, mycolic acids play a vital role in making *M. tuberculosis* resistant to numerous antibiotics and disinfectants, as well as giving it its distinctive waxy look. *M. tuberculosis* has a slow growth rate compared to many other bacteria. The slow growth characteristic contributes to the chronic nature of TB infections. For the bacterial pathogenesis, the bacterium mostly affects the lungs, producing symptoms like fever, night sweats, weight loss, and a chronic cough. The capacity of *M. tuberculosis* to elude host immune responses, endure inside host cells (such macrophages), and create long-lasting infections is largely dependent on its cell wall. In addition, *M. tuberculosis* can survive in airborne droplets, usually from individuals with active TB disease. Therefore, it is crucial to comprehend the makeup and structure of the *M. tuberculosis* cell wall in order to create

novel treatments and vaccinations against the disease, as well as its etiology and mechanisms of antibiotic resistance. [12]

2.3 Antibiotic resistance

Antibiotic resistant *M. tuberculosis* is a significant public health concern worldwide. In general, treatment for TB usually consists of a course of many medicines administered over several months. It is well-known that TB infection is treated with first-line drugs isoniazid and rifampicin. The combination of isoniazid, rifampicin, and pyrazinamide effectively cures *M. tuberculosis* strains for 3 months that are fully drug-susceptible. However, the *M. tuberculosis* may become resistant to antibiotics if they are used improperly or insufficiently. Multi-Drug Resistant TB (MDR-TB) is defined as *M. tuberculosis* that is resistant to at least two of the most effective TB drugs, isoniazid and rifampicin. Furthermore, extensively drug-resistant TB (XDR-TB) is defined as MDR-TB plus resistance to one of the fluoroquinolones and to one of the second-line injectable anti-TB drugs: amikacin, kanamycin, or capreomycin.

Isoniazid resistance checks for mutations in the *katG* and *inhA* genes, while rifampicin resistance checks for *rpoB* gene mutations. The sensitivities of the isoniazid and rifampicin resistance tests are 94.5% and 95.8%, respectively. The specificity is between 99.3% and 98.4%. Using the LPA method, testing for resistance to second-line TB drugs can be done for two groups of drugs: fluoroquinolones and second-line injectable drugs. The test's sensitivity for first-line TB drug resistance is relatively low. The sensitivity of the test is less than that of testing for resistance to first-line TB drugs, where the fluoroquinolones group and the second-line injectable drugs group have a test sensitivity of 86.2% and 87.0%, respectively, while the specificity is 98.6% and 99.5%, according to Sequence. [13,14]

Testing for Rifampicin resistance involves detecting mutations in the *rpoB* gene, whereas detecting Isoniazid resistance involves detecting mutations in two different genes. The *katG* gene is involved in creating the enzyme catalase peroxidase to convert isoniazid from a pro-drug to an active one. This mutation has formed. It is related to high-level resistance; that is, the bacteria will be resistant to isoniazid at a concentration of 1 µg/mL. The *inhA* gene is involved in the synthesis of mycolic acid, which is where isoniazid comes to act. A mutation can led to low-level resistance, indicating that the bacteria has developed resistance. 0.2 µg/mL, but it remains sensitive to Isoniazid at a concentration of 1 µg/mL, indicating that this group can still contemplate using higher doses of Isoniazid for treatment. [15]

Isoniazid is a commonly used drug for treating *M. tuberculosis* infections by inhibiting NADH-dependent enoyl-ACP reductase encoded by *inhA* and prevents mycolic acid synthesis [7]. Isoniazid acts as a prodrug that requires activation by the *M. tuberculosis* catalase-peroxidase enzyme *katG*. Mutations in *katG* and *inhA* genes confer resistance to isoniazid [7]. Infection control is becoming more challenging because of rising drug resistance, especially to the first-line antibiotic isoniazid, and the occurrence of MDR-TB [8]. However, the occurrence of isoniazid-resistant *M. tuberculosis* caused by mutation *inhA* and *katG* has been reported in many countries, including Thailand [16, 17]. It has been reported that the most prominent mutations of Ser315Thr substitution in *katG* and -15 C to T substitution in the regulatory region of *inhA* were detected in antibiotic-resistant *M. tuberculosis* in Thailand [18]. Depending on the *M. tuberculosis* population, different countries may have different mutation patterns and frequencies.

2.4 Tuberculosis situation

According to the results of tuberculosis operations in the fiscal year 2019, there were patients registered for tuberculosis treatment in Thailand. The total number of patients, both new and recurrent, was 87,789, indicating an 84 percent search and treatment coverage rate. Patients with tuberculosis and HIV infection accounted for 6,798 cases, representing 10 percent of those who received treatment. Get tested for HIV. For multidrug-resistant tuberculosis According to estimates from the World Health Organization, Thailand is home to 2,500 patients with drug-resistant tuberculosis (MDR-TB/RR-TB), or 3.6 per 100,000 people. There are 2,500 patients with drug-resistant tuberculosis (MDR-TB/RR-TB), or 3.6 per 100,000 people. MDR-TB/RR-TB drug-resistant TB was found in 1.7% of new TB patients and 10% of patients with a history of previous TB treatment. The work's results revealed that the Ministry of Public Health registered 1,221 MDR-TB/RR-TB drug-resistant tuberculosis patients with laboratory confirmation for treatment and another 1,095 for treatment. The Ministry of Public Health has also issued a statement. The Ministry of Public Health has declared very severe multidrug-resistant tuberculosis (XDR-TB) a dangerous infectious disease. The Communicable Diseases Act of 2015 mandates the implementation of legal measures to bolster efforts to prevent and control extremely severe multidrug-resistant tuberculosis. In 2020, cases of multidrug-resistant tuberculosis were discovered and reported. 15 cases were very severe. [19]

The tuberculosis situation in Thailand in 2016–2020 found that tuberculosis patients registered for treatment in the NTIP program tend to increase. It was found that there were

79,033 new and recurrent tuberculosis patients registered for treatment in 2018, 81,907 cases in 2019, and 81,268 cases in 2020 (information as of June 28, 2021). From drug resistance surveillance in Thailand in 2018, it was found that 0.8% of new patients were found to have MDR-TB/RR-TB, and 13% of patients with a history of prior treatment were found to have MDR-TB/RR-TB. Results of drug-resistant tuberculosis operations in Thailand found that the detection and registration of treatment for drug-resistant tuberculosis (MDR-TB/RR-TB) in 2015–2020 increased from 2015. It can be seen that MDR-TB-resistant tuberculosis patients and RR-TB that have been detected have increased registration for treatment. In 2020, 1,237 cases of MDR-TB/RR-TB drug-resistant TB were detected, and 1,142 cases were registered for treatment [19].

The upper southern region of Thailand is a densely populated area. There has been a high incidence of new tuberculosis cases. The Department of Disease Control, Ministry of Public Health, provides data from the NTIP program (National Tuberculosis Information Program). It was found that the coverage rate for registration of new and recurrent TB patients (All Form) (TB treatment coverage) for the year 2020-2022 in Health Zone 11 was 76.70%, 68.70%, and 75.50%; at the national level, it was 78.40%, 68.50%, and 69.40%. The treatment success rate for new and recurrent TB patients (all forms) (TB Success Rate) in 2019–2021 in Health Zone 11 was 88.09%, 87.79%, and 86.58%; at the national level, it was 86.35%, 85.61%, and 84.54%. The death rates for new and recurrent TB patients (all forms) in Health Zone 11 were 7.58%, 7.58%, and 8.94% in 2019–2021, while at the national level, they were 7.95%, 8.23%, and 8.44%. The drug deficiency rates for new and recurrent TB patients (all forms) (TB loss follow-up) in 2019–2021 in Health Zone 11 were 3.58%, 3.58%, and 3.45%, while at the national level, they were 4.46%, 4.67%, and 4.87%. The Disease Prevention and Control Office Region 11, Nakhon Si Thammarat, is responsible for controlling and preventing tuberculosis disease, and the medical laboratory is in charge of diagnosing tuberculosis in this area of responsibility.

Therefore, the objective of this study was to retrospectively investigate the occurrence and characteristics of drug resistance in *Mycobacterium tuberculosis* associated with mutations in the *inhA* and *katG* genes in *M. tuberculosis* isolates from patients in the southern region of Thailand during the fiscal year 2020-2022, which included 7 provinces: Chumphon, Surat Thani, Nakhon Si Thammarat, Phuket, Krabi, Phang Nga, and Ranong.

Chapter 3

Materials and Methods

3.1 Ethical approval

A retrospective dataset of TB during the fiscal years 2020–2022 was received from the Office of Disease Prevention and Control Region 11st, Nakhon Si Thammarat, Thailand. The laboratory data were secondary data from a TB laboratory performed at the Office of Disease Prevention and Control Region 11st, Nakhon Si Thammarat, Thailand, under the Ministry of Public Health Thailand. All study procedures were approved by the Ethics Scientific Committee of Boromarajonani College of Nursing, Nakhon Si Thammarat, Thailand (Ref No. Exc-10/2566).

3.2 Study period, location, and sample collection

All data for this study were collected in fiscal years 2020 and 2022. In Thailand, the fiscal year runs from 1st October to 30th September of the following year. Therefore, fiscal year 2020 spanned from October 1, 2019, to September 30, 2020. The approach for fiscal years 2021 and 2022 was carried out as previously explained. The Office of Disease Prevention and Control Region 11st collected the clinical samples associated with TB from hospitals located in seven provinces, including Nakhon Si Thammarat, Surat Thani, Phuket, Krabi, Chumphon, Ranong, and Phang Nga provinces (Figure-4). The collected samples are shown in Table-1.

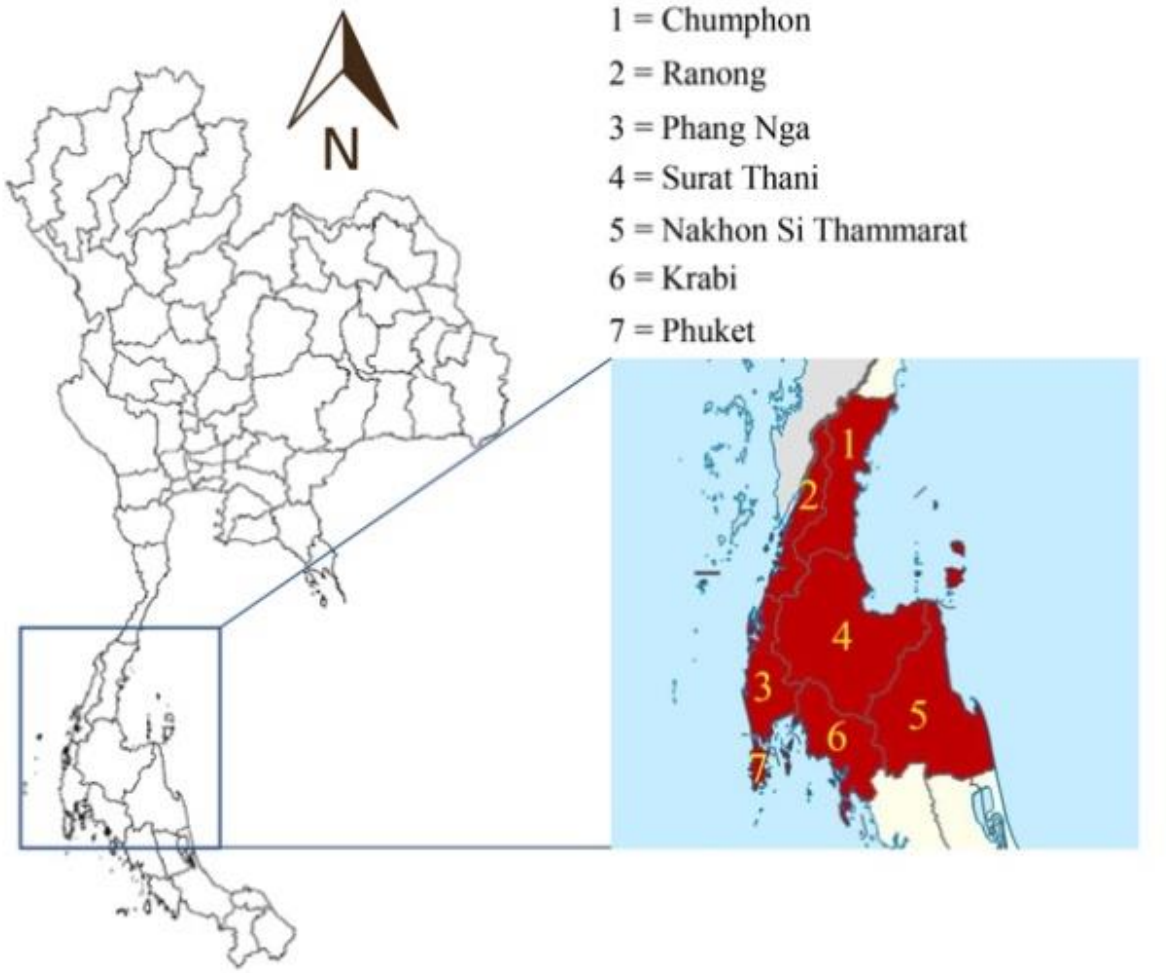


Figure-4: Locations in upper Southern Thailand. All samples of Tuberculosis from this area are sent to Office of Disease Prevention and Control Region 11st, Nakhon Si Thammarat, Thailand. [Source of the map: https://cleverlearn-hocthongminh.edu.vn]

Table-1: Sample collection.

Samples	Numbers of samples (n)/Year			Total (n)
	2020	2021	2022	
Abdominal fluid	7	1	-	8
Ascitic fluid	2	1	-	3
Bronchoalveolar lavage	131	-	6	137
Blood	34	11	2	47
Bone marrow	19	1	-	20
Bone/Joint	5	1	-	6
Cerebrospinal fluid	64	5	-	69
Gastric lavage/ washing	15	2	-	17
Joint fluid	5	-	1	6
Lymph node	27	1	-	28
Peritoneal dialysis fluid	8	-	-	8
Pericardial fluid	9	1	1	11
Peritoneal fluid	7	-	-	7
Pleural effusion/ fluid	94	13	9	116
Pus	58	14	6	78
Skin	1	-	-	1
Sputum/Suction tube (sputum)	6,959	7,054	4,482	18,495
Stool	8	-	-	8
Synovial fluid	2	-	-	2
Tissue	39	3	1	43
Urine	45	2	-	47
Other	26	-	3	29
Total	**7,565**	**7,110**	**4,511**	**19,186**

Source: The Office of Disease Prevention and Control Region 11st collected the clinical samples associated with TB from hospitals located in seven provinces, including Nakhon Si Thammarat, Surat Thani, Phuket, Krabi, Chumphon, Ranong, and Phang Nga provinces.

3.3 Detection, culture, and identification of *M. tuberculosis*

The detection, culture, and identification of *M. tuberculosis* from the collected samples were performed as shown in the overall flowchart of this study in Figure-5. Patients with abnormal X-ray chests were screened for the presence of acid-fast bacilli (AFB) in the clinical samples. For AFB-positive, the samples were recorded according to the criteria as non-*M. tuberculosis* (NTM, *Mycobacterium* spp. without *M. tuberculosis*), followed up, multidrug resistance, pre-XDR-TB, XDR-TB, diagnosis, EP, followed up on the treatments, relapse, treatment after loss failure (TALF), treatment after default (TAD), and new case. All the samples as described above, except the new case, which is non-resistant to isoniazid and rifampicin, were then cultured in Middlebrook 7H9 liquid media associated with BACTEC™ MGIT™ 960 (MGIT 960) (Becton Dickinson, Shannon, Ireland) and Lowenstein-Jensen medium (Nonthaburi, Thailand) and incubated at 37 ± 1°C for 6–8 weeks. The bacteria were identified as *M. tuberculosis* using a BD BACTEC™ MGIT™ and BD MGIT™ TBc identification test (TAUNS Laboratory, Japan). For negative AFB, the samples were recorded according to the criteria described above. The samples, including NTM, EP, follow-up, MDR-TB, pre-XDR-TB, and XDR-TB, were cultured as described above, while the samples groups of diagnosis, relapse, TALF, and TAD were further detected for the presence of the *M. tuberculosis* genome by GeneXpert (Cepheid, Solna, Sweden).

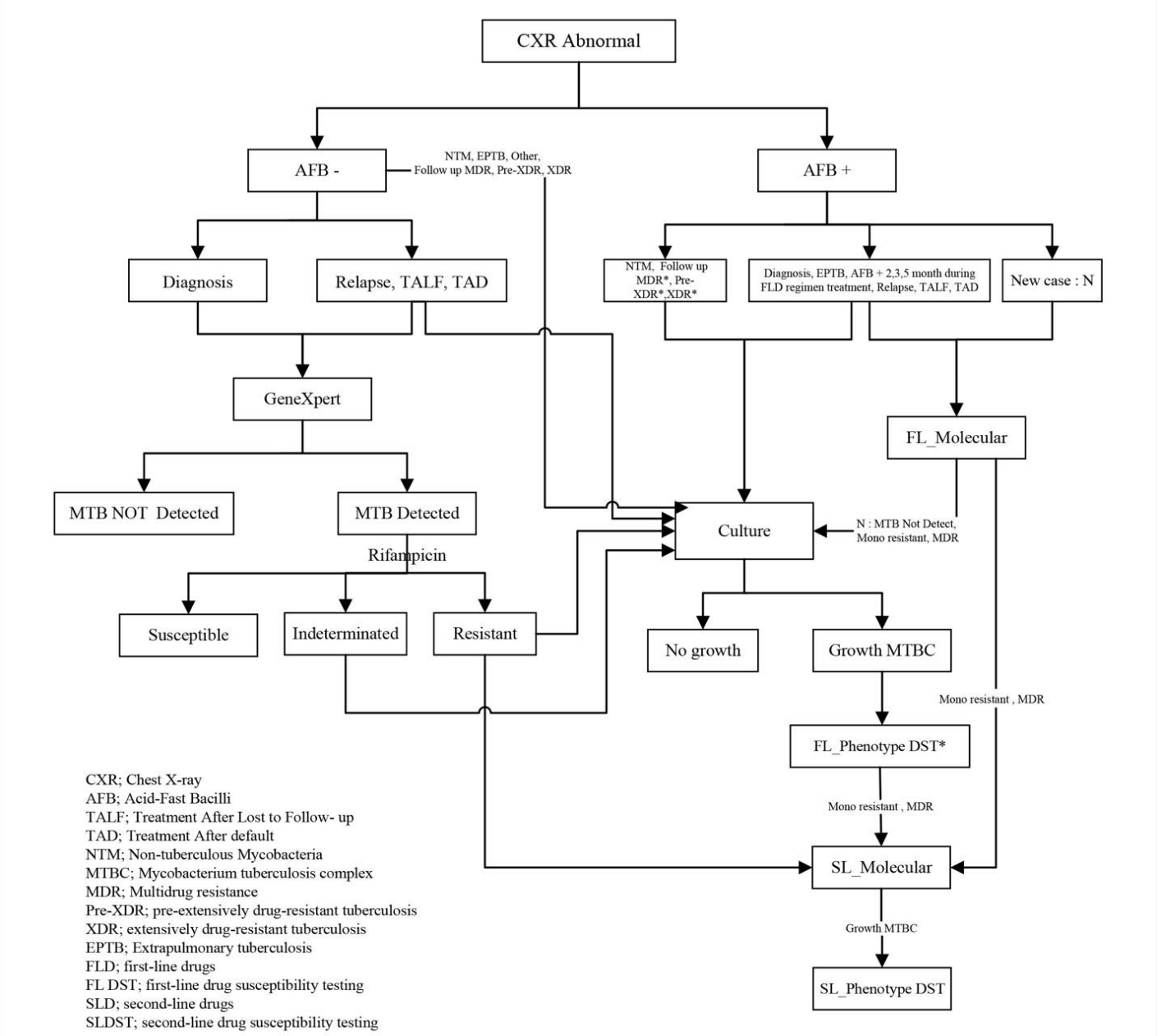


Figure-5: Flow chart of the detection of *Mycobacterium tuberculosis* and antibiotic susceptibility from the samples submitted to Office of Disease Prevention and Control Region 11st, Nakhon Si Thammarat, Thailand.

3.4 Antimicrobial susceptibility

The antibiotic susceptibility of *M. tuberculosis* clinical isolates was investigated using MGIT 960 (Becton Dickinson, Shannon, Ireland). The antibiotics primarily contain isoniazid, rifampicin, ethambutol, and streptomycin. The bacterial colonies were mixed in tubes containing glass beads. Then, the sample was adjusted for turbidity to the 0.5 MacFarland standard. The samples were then diluted in sterile water (1:5 and 1:100). The sample at 1:5 was added to the tube containing the antibiotics to investigate antibiotic susceptibility. It was

noticed that the bacterial suspension at 1:100 was used as the growth control because it could present the intense luminescence that presenting the growth positive results. The samples were incubated at 37 ± 1°C for 13 days. The results were interpreted as sensitive (S), resistant (R), and invalid (I). In addition, the sample tubes that were contaminated with other organisms were interpreted as contaminated (C). MDR-TB is defined as resistance to at least two of the first-line anti-TB drugs, including isoniazid and rifampicin. XDR-TB was defined as MDR-TB in the presence of the antibiotic resistance genes *gryrA* or *gryrB* (fluroquinolone) and *res* or *eis* (aminoglycoside). In addition, pre-XDR-TB was defined as MDR-TB with resistance plus at least fluoroquinolone or second-line injectables.

3.5 Detection of mutations associated with *inhA* and *katG* genes

All antibiotic-resistant *M. tuberculosis* mutations associated with *inhA* and *katG* genes were then detected by Line probe assay (Genotype® MTBDRplusVer2.0 [HainLife Science, Nehren, Germany]) and real-time polymerase chain reaction (PCR) as previously described by WHO [6]. RNA was extracted using a commercial Qiagen kit (Nehren, Germany). Only positive cultures (MGIT 960, LJ Medium) or AFB-positive samples were used for the test. Following the manufacturer's instructions, the process comprised DNA extraction, multiplex amplification using biotinylated primers, and DNA reverse hybridization. Mutations in the *inhA* regulatory area and *katG* gene, which confer resistance, were evaluated.

3.6 Statistical and spatial analyses

The collected data were analyzed using descriptive statistics. All statistical analyses were performed using the R programming language version 4.3.2 (https://www.r-project.org). In addition, data on population demography and the number of TB-infected cases were used to calculate the incidence classified by years and area. The calculation was performed using Microsoft Excel 2021, and the occurrence was presented as per 100,000 risk person-years. The incidence was calculated as;

Incidence = ([Number of new cases]/ [Number of populations at risk]) × 100,000

Information on TB incidence was used to create the spatial distribution map using Quantum GIS desktop 3.22 software (https://www.qgis.org). The grading of incidence per area followed the Jenks Natural Break method with five classes. The classes of incidence per area were classified as none, low incidence, moderate incidence, high incidence, and extremely high incidence.

Chapter 4

Results

4.1 Sample collection and identification of *M. tuberculosis*

Samples were collected from patients who were suspected of having TB as diagnosed by doctors and other medical diagnoses. A total of 19,186 samples associated with TB were sent to the Office of Disease Prevention and Control Region 11st, Nakhon Si Thammarat, Thailand. The results revealed that 51% of the samples were received from patients located in Nakhon Si Thammarat province, followed by Surat Thani province (20%) and Krabi province (8%), respectively (Figure-6a). During the years 2020–2022, the number of samples associated with TB decreased over time (Table-1 and Figure-6b). However, the total number of samples in Nakhon Si Thammarat province in 2021 was higher than those in 2020 and 2022. It was noticed that the highest number of samples collected from the patients was sputum, followed by bronchoalveolar lavage and pleural effusion/ fluid.

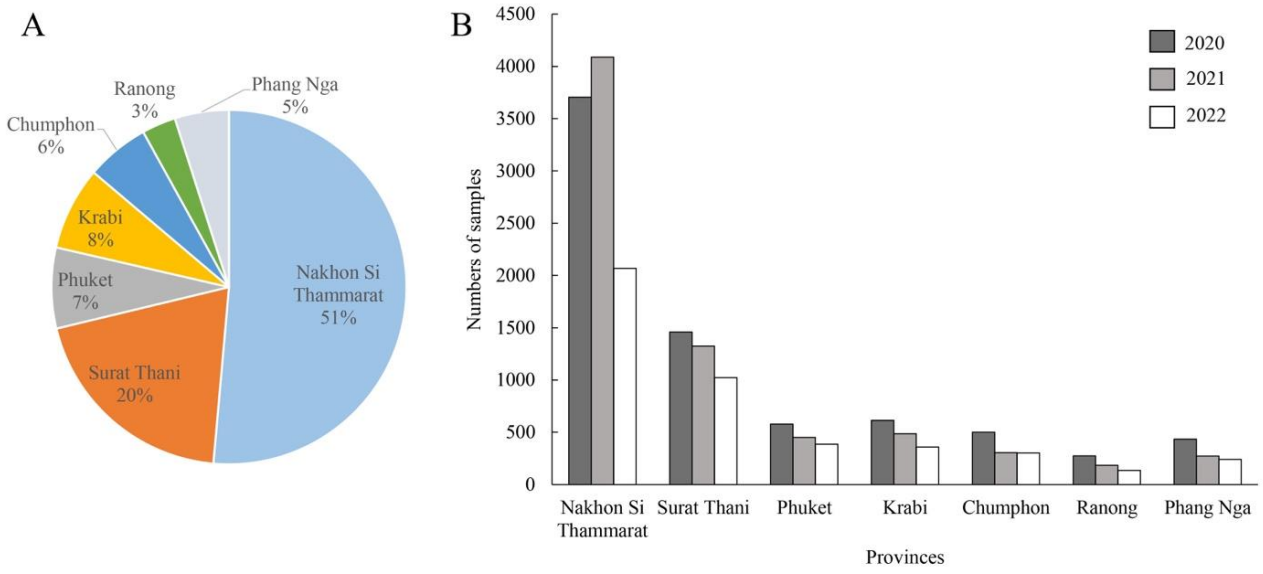


Figure-6: (a) Distribution of samples associated with Tuberculosis in different area controlled by Office of Disease Prevention and Control Region 11st, Nakhon Si Thammarat, Thailand. (b) Numbers of the samples in different areas in the fiscal years 2020-2022.

4.2 Spatial analyses

Regarding the spatial distribution of TB-infected cases, the incidence of TB was high in the province, which had a moderate to high density of population (Figures-7a and b). The highest average incidence of TB in this study was found in Phuket province (9.75/100,000 risk person-year). In contrast, Phang Nga province had the lowest average TB incidence (5.72/100,000 risk person-years). Mainly, the incidence of TB in this region was found to be high. The provinces with high incidence were Chumphon (7.78/100,000 risk person-year), Surat Thani (7.57/100,000 risk person-year), Krabi (8.08/100,000 risk person-year), and Phuket. The incidence of TB in each province was found to be different (Figures-4c-e). Interestingly, the incidences of TB in Chumphon, Krabi, and Nakhon Si Thammarat provinces were increasing; in contrast, the incidences in Ranong and Phang Nga provinces slightly decreased annually.

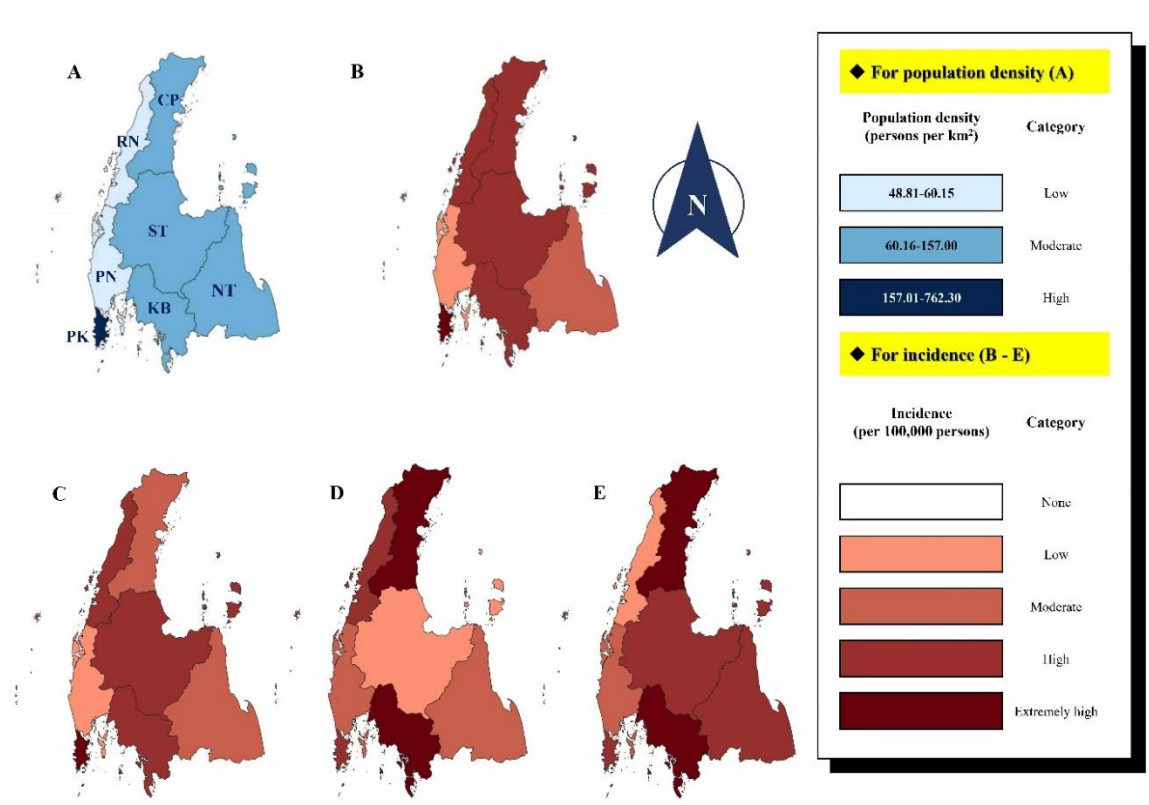


Figure-7: The spatial distribution of tuberculosis (TB) infected cases in the studied area including (a) map of studied provinces and the density of the population, (b) map of average TB incidence by area between 2020-2022, (c) map of TB incidence by area in 2020, (d) map of TB incidence by area in 2021, and (e) map of TB incidence by area in 2022. The map is generated using Quantum GIS version 3.34 software. (Abbreviations contain CP: Chumphon; RN: Ranong; ST: Surat Thani; PN: Phang Nga; KB: Krabi; NT: Nakhon Si Thammarat; PK: Phuket).

4.3 Antibiotic resistance of clinical isolates of *M. tuberculosis*

The antibiotic resistance of the clinical isolates of *M. tuberculosis* was determined based on the first- and second-line anti-TB drugs. The results demonstrated that most clinically relevant *M. tuberculosis* isolates were resistant to isoniazid (Table-2,3). The detected isoniazid resistance was 394, 255, and 179 cases in 2020, 2021, and 2022, respectively. Resistance to rifampicin in 2020, 2021, and 2022 was 25, 9, and 12 cases, respectively. The results showed that 46, 24, and 29 isolates possessed multidrug resistance in the years 2020, 2021, and 2022, respectively. Pre-XDR-TB and XDR-TB of the clinically relevant *M. tuberculosis* isolates were detected during the time of this study. An increase in mono-resistance, MDR-TB, pre-XDR-TB, and XDR-TB was observed in fiscal year 2022 compared with fiscal years 2020 and 2021.

Table-2: Antibiotic resistance from sample collection.

The type of resistance	**Sample collection**	**Year**		
		2020 (n = 2744)	**2021 (n = 1887)**	**2022 (n = 670)**
Isoniazid		**394**	**255**	**179**
	Bronchoalveolar lavage (BAL), culture bottle; BAL	4	-	1
	CSF	-	1	-
	Lymph Node, culture bottle; Lymph Node	1	-	-
	Pus, culture bottle; pus	2	-	-
	Sputum, Suction Tube (Sputum), culture bottle; Sputum	384	254	178
	stool	1	-	-
	Tissue, culture bottle; tissue	1	-	-
	urine	1	-	-
Rifampicin		**25**	**9**	**12**
	Sputum, Suction Tube (Sputum), culture bottle; Sputum	25	9	12
MDR-TB		**46**	**24**	**29**
	Sputum, Suction Tube (Sputum), culture bottle; Sputum	44	23	29
	Pus, culture bottle; pus	1	1	-
	other	1	-	-
Pre-XDR-TB		**7**	**1**	**4**
	Sputum, Suction Tube (Sputum), culture bottle; Sputum	7	1	4
XDR-TB		**1**	**2**	**1**
	Sputum, Suction Tube (Sputum), culture bottle; Sputum	1	2	1
Total		**473**	**291**	**225**

Table-3: Antibiotic resistance of clinical isolates of *M. tuberculosis.*

The type of resistance	Antibiotics	Year		
		2020 (n = 2744) (%)	2021 (n = 1887) (%)	2022 (n = 670) (%)
Mono-resistance	Isoniazid	394 (14.36)	255 (13.51)	179 (26.72)
	Rifampicin	25 (0.91)	9 (0.48)	12 (1.79)
MDR-TB	Isoniazid and rifampicin	46 (1.68)	24 (1.27)	29 (4.33)
Pre-XDR-TB	Isoniazid, rifampicin, and fluoroquinolones[a] or second-line injectables[b]	7 (0.26)	1 (0.05)	4 (0.60)
XDR-TB	Isoniazid, rifampicin, fluoroquinolones, and second-line injectables	1 (0.04)	2 (0.11)	1 (0.15)

[a]Fluoroquinolone contain ofloxacin, levofloxacin, moxifloxacin. [b]Second-line injectables containing amikacin, capreomycin, and kanamycin. *M. tuberculosis=Mycobacterium tuberculosis*, MDR-TB=multi-drug resistance, XDR-TB=Extensively drug-resistant

4.4 Detection of *inhA* and *katG* mutations

The detection of mutations in both *inhA* and *katG* genes associated with isoniazid resistance in *M. tuberculosis* was investigated. As detected by the line probe assay, 191 isolates showed an *inhA* mutation, whereas 248 isolates presented a *katG* gene mutation in the year 2020 (Table-4). In 2021, the detection of each *inhA* and *katG* mutation was 139 and 179 isolates, respectively. In addition, 43 samples with the *inhA* mutation and 55 isolates with the *katG* mutation were observed in the clinical isolates of *M. tuberculosis* collected in 2022. Furthermore, three isolates collected in 2020 and one isolate collected in 2021 possessed both *inhA* and *katG* mutations.

The detection of *inhA* and *katG* mutations associated with isoniazid resistance in *M. tuberculosis* is further determined by real-time PCR if the results of the line probe assay are invalid. As shown in Table-4, one isolate detected in 2020 showed both *inhA* and *katG* mutations. In 2021, three isolates possessed each *inhA* and *katG* mutation. It is interesting to note that 62 isolates were detected with the *inhA* mutation and 65 isolates with the *katG*

mutation in 2022. Furthermore, one isolate showed both *inhA* and *katG* mutations.

Table-4: Detection of antibiotic-resistant genes in clinical samples of TB.

Methodology/year	**No. of isolates carrying antibiotic-resistant genes**		
	***inhA* alone**	***katG* alone**	***inhA* + *katG***
Line probe assay			
2020 (n = 442) (%)	191 (43.21)	248 (56.11)	3 (0.68)
2021 (n = 319) (%)	139 (43.57)	179 (56.11)	1 (0.31)
2022 (n = 98) (%)	43 (43.88)	55 (56.12)	0
Realtime PCR			
2020 (n = 3) (%)	1 (33.33)	1 (33.33)	1 (33.33)
2021 (n = 6) (%)	3 (50.00)	3 (50.00)	0
2022 (n = 128) (%)	62 (48.44)	65 (50.78)	1 (0.78)

TB=Tuberculosis, PCR=Polymerase chain reaction

Chapter 5

Conclusion

This study revealed a retrospective prevalence of TB and antibiotic-resistant *M. tuberculosis* isolated from patients with TB located in upper Southern Thailand during the years 2020–2022. The results showed that 51% of the samples were obtained from patients located in Nakhon Si Thammarat, followed by Surat Thani provinces. However, the highest incidence of TB in this study was found in Phuket province. Furthermore, 99 isolates were MDR-TB, whereas four isolates were XDR-TB. In addition, antimicrobial resistance associated with mutations in the *inhA* and *katG* genes was detected in the isolates. These findings contribute to the understanding of the occurrence and characteristics of antibiotic-resistant TB that could lead to use as data for preventing drug-resistant -TB.

Discussion

TB, caused by *M. tuberculosis,* remains a significant global health concern. The present study revealed the prevalence of TB as well as the detection of multidrug resistance associated with *inhA* and *katG* genes in patients with *M. tuberculosis* in upper Southern Thailand. The data were provided by the Office of Disease Prevention and Control Region 11st, Nakhon Si Thammarat, Thailand, during the COVID-19 pandemic (2020–2022). The results revealed that most of the samples were obtained from patients located in Nakhon Si Thammarat province. This may be due to the location of the office in the province as well as the largest population of hospitals and people compared with other provinces in the upper Southern region of Thailand. However, the highest average incidence of TB in this study was in Phuket province, which may have affected the population density in the province. Interestingly, the incidences of TB in Chumphon province, Krabi province, and Nakhon Si Thammarat province increased by the year, whereas in Ranong province and Phang Nga province, they slightly decreased by the year. In 2018, 186 patients with TB were recorded and diagnosed at Sirindhorn Hospital in Bangkok, the capital city of Thailand [20]. It has been reported that 466/1002 (46.5%) participants had a latent TB infection, as detected in Klong Prem Central Prison, a maximum-security prison in Bangkok, Thailand [21]. At King Chulalongkorn Memorial Hospital in Thailand,

there were 164 cases of TB/100,000 workers reported by Mingchay *et al.* [11]. Moreover, most of the patients with positive results were the young age group [22]. The incidence of TB in other Asian countries has been documented. Southern Asia (22%) showed the highest prevalence of TB, followed by Eastern Asia (18%) and South-east Asia (16%), as described by a systematic review and meta-analysis of articles published from 2012 to 2021 [23].

This study collected data on TB from 2020 to 2022, which was the period of the COVID-19 pandemic. The results revealed that the number of samples associated with TB in Upper Southern Thailand decreased over the study period. This finding was similar to the incidence of TB in several nations during the pandemic of COVID-19 [24]. Furthermore, the WHO has reported that the COVID-19 pandemic has negatively impacted TB case notifications compared with data from 2019 and 2020 [25]. However, our data indicated a rise of cases during COVID-19 peak times in 2020, which is 7565 cases, and in 2021 after COVID, it was reduced to 4511 cases. This may have resulted from human behavior during the COVID-19 pandemic. People stay home and wear a mask, which results in a reduction in direct contact with TB patients. In addition, Thai people still wear masks after COVID-19 spread.

Antibiotic combinations can be used to treat TB in humans. Isoniazid is one of the first drugs of choice for *M. tuberculosis* treatment because it inhibits mycolic acid biosynthesis. However, the occurrence of isoniazid-resistant *M. tuberculosis* caused by mutation *inhA* and *katG* has been reported in many countries, including Thailand [16, 17]. From the study Prevalence and associated risk factors of drug-resistant tuberculosis in Thailand: Results from the fifth national anti-tuberculosis drug resistance survey, it was found that the burden of isoniazid resistance was highest, with 9.7% of new TB cases and 21.3% of previously treated TB cases. Patients with resistance to rifampicin (RR-TB) were detected in 1.3% of new TB patients and 19.6% of previously treated TB patients. Multidrug-resistant TB (MDR-TB) accounted for 0.8% of new TB cases and 13.0% of previously treated TB cases [17]. It is well known that the *inhA* gene encodes for enoyl acyl reductase and is involved in mycolic acid biosynthesis, whereas the *katG* gene encodes catalase-peroxidase, which is necessary for the activation of the prodrug isoniazid [7]. Therefore, the detection of the mutation of two genes

in the clinical isolates may estimate isoniazid resistance in the area. According to the results, isoniazid was the most resistant antibiotic found in clinical *M. tuberculosis* isolates. This finding was similar to previous studies by Rudeeaneksin *et al.* [18] on the prevalence of antibiotic-resistant *M. tuberculosis* in Thailand and other countries like Indonesia [26]. It has been reported that the most prominent mutations of Ser315Thr substitution in *katG* and −15 C to T substitution in the regulatory region of *inhA* were detected in antibiotic-resistant *M. tuberculosis* in Thailand [18]. Depending on the *M. tuberculosis* population, different countries may have different mutation patterns and frequencies.

Isoniazid is eliminated from the body by arylamine N-acetyltransferase type 2 (NAT2), which exhibits genetic polymorphisms that can be classified into rapid acetylators, intermediate acetylators, and slow acetylators. Therefore, in patients receiving the same dose of isoniazid, those who are slow acetylators will have higher blood levels of the drug, increasing the risk of adverse effects such as drug-induced hepatotoxicity. In contrast, patients who are rapid acetylators are more likely to experience treatment failure due to lower blood concentrations of isoniazid. In the future, allele testing to determine acetylator activity may play a significant role in selecting the appropriate isoniazid dosage for individual patients [27].

It was noticed that the data analysis in this study was performed according to the standard procedure for TB recommended by the Office of Disease Prevention and Control Region, Thailand. Recently, the definitions of pre-extensively and XDR-TB-TB have been updated by the WHO in 2021 [28]. However, the data collection and analysis of TB at the Office of Disease Prevention and Control Region 11st, Thailand, have been investigated using the old standard procedure available in Thailand [15]. This study revealed the detection of pre-XDR-TB and MDR-TB of clinically relevant *M. tuberculosis* isolates in the years 2020–2022. It was noticed that the percent resistance, including mono-resistance, MDR-TB, pre-XDR-TB, and XDR-TB, of the clinically isolated *M. tuberculosis* isolates increased in 2022 compared with the previous years. Furthermore, six of the clinically relevant *M. tuberculosis* isolates possessed multidrug resistance, presenting both *inhA* and *katG* mutations during the years 2020–2022. The results of this study indicate that diagnosing tuberculosis drug resistance, specifically regarding rifampicin, is not suitable for the upper southern region due to the high levels of isoniazid resistance found in the analyzed data. Therefore, in this region, isoniazid

resistance testing should be prioritized. To enhance diagnostic accuracy, both genotypic and phenotypic testing methods should be utilized for all patients undergoing tuberculosis treatment. Additionally, to improve treatment efficiency and to prevent and control the spread of drug-resistant tuberculosis, further studies on the mutation sites in the *inhA* and *katG* genes that affect isoniazid resistance at different levels are necessary.

However, it is important to note that testing for isoniazid resistance alone does not capture all characteristics of resistance expression. Some patients may show no detectable resistance to isoniazid yet still fail to respond to tuberculosis drug treatment, potentially due to side effects from tuberculosis drug regimens, particularly isoniazid. For these patients, allele testing for the acetylator activity of NAT2 should be conducted to determine its potential impact on isoniazid metabolism, enabling the optimization of drug concentrations in their treatment plans.

In conclusion, the use of appropriate laboratory diagnostic tests significantly enhances treatment effectiveness, helps prevent and control tuberculosis, and can mitigate the further spread of drug resistance in tuberculosis.

Limitation

The limitation of this study was the collection of samples received from hospitals under the Office of Disease Prevention and Control Region 11st, Nakhon Si Thammarat, Thailand. The study encompasses 87 public healthcare facilities and 7 private healthcare facilities, representing 88.68% of the total 106 entities. As a result, the samples in this study did not include all patients in Upper Southern Thailand.

Authors' Contributions

PK, PS, AB, and WM: Conceived and designed the study. PK, AB, SS, PW, and BB: Performed the study. PK, PS, AB, and WM: Analyzed and interpreted the data. PK, PS, AB, and WM: Performed statistical analysis. VN and MLP: Supported analysis tools. PK, PS, VN, MLP, and WM: Wrote the manuscript. PK, PS, VN, MLP, and WM: Revised the manuscript. All authors have read, reviewed, and approved the final manuscript.

Acknowledgments

This work was funded by the Center of Excellence in Innovation of Essential Oil and Bioactive Compounds (Grant no. WU-COE-65-05), Walailak University, Thailand. The authors thank the staff at the Office of Disease Prevention and Control region 11st, Nakhon Si Thammarat, Thailand, for laboratory services and sample collection. We would like to thank One Health Research Center, Walailak University, Thailand, for their support.

Competing Interests

The authors declare that they have no competing interests.

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