Research Article

Prevalence of drug-resistant recurrent tuberculosis and new multidrug-resistant tuberculosis mutations detection in Can Tho, Vietnam

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ABSTRACT

Tuberculosis (TB) has been a threat to world health for decades. Multidrug-resistant TB (MDR-TB) is adding to the burden of disease and hindering the development of countries. This study aimed to identify the drug-resistant rate in patients with recurrent pulmonary TB and detect rifampicin resistance mutations in the *rpoB* gene in Can Tho, Vietnam. In this descriptive cross-sectional study, clinicians diagnosed the patients with recurrent pulmonary TB and were hospitalized. Smear-positive sputum specimens (n=246) were collected to determine the rate of drug resistance. To identify mutations in the *rpoB* gene which are likely to be associated with rifampicin resistance in MDR-TB, we sequenced 40 isolates of Mycobacterium tuberculosis collected between 2012 and 2014; mainly MDR-TB (n=40; 95.2%) and rifampicin-sensitive TB (n=2; 4.8%). The rate of drug-resistant pulmonary tuberculosis was 63.8%, and the rate of MDR-TB accounted for 23.2%. The mutations in *rpoB* were predominantly in codons 531 (27.5%) and 523 (17.5%), with rare occurrences of S522A (2.5%) and A532P (2.5%). Noticeably, there was a substitution mutation in codon 532, and a mutant strain of tuberculosis at seven codons in the *rpoB* gene had been detected. In conclusion, this study provided drug-resistant characteristics in patients with recurrent pulmonary TB and mutations of MDR-TB in Can Tho, Vietnam. The results show a mutation in codon 532 of the *rpoB* gene and a mutant strain of tuberculosis at all seven codons. These are remarkable and promising results for further studies and clinical applications.

Keywords:

Tuberculosis, MDR-TB, RRDR, Drug resistance, Multidrug-resistant

1. INTRODUCTION

Until antibiotics were discovered, tuberculosis was an incurable disease. However, the threat posed by tuberculosis bacteria has gradually returned because of the emergence of drug resistance. Globally, WHO estimated that about 10 million people fall ill with tuberculosis (TB) by 2020. In 2021, WHO reported TB is the 13th leading cause of mortality worldwide and the second leading infectious killer after COVID-19¹. Multidrug-resistant tuberculosis (MDR-TB) continues to be a public health concern and a threat to health security¹. In 2020, just about one-third of patients with MDR-TB received treatment. Many countries have been bearing the burden of the high expense of tuberculosis treatment. For low-income countries, the predicted median cost per 6 months of therapy was US\$315.30 (95% CI US\$222.60-US\$417.20)². Vietnam is one of the 30 countries globally with the highest TB burden¹, profoundly affecting the country. Identifying the drug-resistant rate of tuberculosis is essential, especially in areas with high tuberculosis rates, including the Southeast and the Mekong Delta³. Recently, many studies have been published that found rates of TB resistance in Ha Noi, Vietnam (2019)⁴, India (2020)⁵, Dalian, China (2021)⁶. However, few studies have been conducted to establish the proportion of drug resistance in Can Tho City (Mekong Delta). Determining the prevalence of drugresistant TB bacteria by geographical area is essential for

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early detection of drug-resistant TB⁷ and patients' treatment.

Antituberculosis drugs are a double-edged sword. Although they kill TB bacteria, they give rise to drug resistance. Many factors related to drug resistance prevent the drug concentrations from being high enough to kill the TB bacteria. TB bacteria that are not entirely eradicated will mutate to become drug-resistant. The resistance to antituberculosis agents varies even within the same nation⁸. The detection of drug-resistant mutations of TB bacteria has been studied globally, including in Vietnam. Our study was conducted to contribute to the clarification of drug-resistant mutations in MDR-TB. Overall, this study aims to determine the prevalence of antituberculosis drug resistance in patients with recurrent pulmonary tuberculosis and detect the rifampicinresistant gene mutation in MDR-TB in Can Tho, Vietnam.

2. MATERIALS AND METHODS

2.1. Study participants

We conducted a study on patients with recurrent pulmonary tuberculosis being treated at Can Tho hospital of Tuberculosis and Lung Diseases. Study subjects were patients with a confirmed diagnosis of recurrent pulmonary tuberculosis treated at the hospital and their sputum samples. The samples were collected and analyzed over four years. After determining the participants' drug resistance rate, the following study was conducted on 40 strains selected from the antibiotic susceptibility testing. Antibiogram results showed 40 strains of MDR/RR-TB (rifampicin- and isoniazid-resistant). The importance of antibiotic sensitivity testing has been emphasized in study⁹. Therefore, we selected the exclusion criterion as patients with pulmonary tuberculosis who did not have indications or results of antibiograms.

2.2. Study design

This study is a cross-sectional descriptive study with analysis. The study was conducted to determine the rate of drug resistance in patients with recurrent pulmonary tuberculosis. We regularly collected data to analyze and detect resistance mutations in MDR-TB patients.

2.3. Data collection and sampling

2.3.1. Prevalence of drug-resistant recurrent pulmonary TB

Simple random sampling with equal probability and the formula for calculating sample size with α =0.05, Z_{0.975}=1.96, *p*=0.19 and d=0.05.

There were 246 sputum samples of the patients

following the sampling criteria. Direct sputum smear microscopy had shown positive for acid-fast bacilli (AFB+), and we also found positive bacterial cultures in the samples. Meanwhile, the antibiogram results with first-line antituberculosis agents were also performed.

Inclusion criteria of study participants

Recurrent pulmonary TB patients were diagnosed based on classic symptoms, evidence of parenchymal infection on radiographs, and medical history of pulmonary TB. Sputum samples were satisfactory after staining, direct smear microscopy, and culture on Lowenstein Jensen (L J) medium. All the patients were managed according to the diagnostic and treatment guidelines of Vietnam's Ministry of Health on National Tuberculosis Prevention and Control Program.

Exclusion criteria of study participants

Patients with the following characteristics: no antibiogram results, do not agree to participate in the study and HIV co-infection, diabetes, etc.

2.3.2. The rifampicin-resistant MDR-TB gene mutations

The 40 strains that showed first-line antituberculosis agents with antibiotic susceptibility testing results were selected. The participants in the study were hospitalized and treated at the Can Tho hospital of Tuberculosis and Lung Diseases. Then the process of sputum sampling and culturing was carried out at the microbiology department of the hospital.

Inclusion criteria of study participants

We selected 40 multidrug-resistant tuberculosis strains by the antibiogram results conducted on the L J medium. All the patients were managed according to the diagnostic and treatment guidelines of Vietnam's Ministry of Health on National Tuberculosis Prevention and Control Program.

Exclusion criteria of study participants

Patients with the following characteristics: no antibiogram results, do not agree to participate in the study and HIV co-infection, diabetes, etc.

Criteria of drug resistance were developed according to Vietnam's National Tuberculosis Prevention and Control Program in Can Tho city. Resistance and sensitivity results were recorded based on the results of the above program according to the standards of Vietnam's Ministry of Health.

2.4. Methods for determining the rate of drug resistance in recurrent pulmonary tuberculosis patients

At Can Tho hospital of Tuberculosis and Lung Disease, the patients were diagnosed with pulmonary tuberculosis with classic clinical symptoms, chest X-ray, and medical history of pulmonary TB. After sampling, the sputum samples were taken to the hospital's laboratory. Physicians indicated antibiotic susceptibility testing, direct sputum smear microscopy, and bacterial culture.

Then, the samples were sent to Pham Ngoc Thach hospital of Tuberculosis and Lung Disease to identify Mycobacteria by biochemical tests and measure the susceptibility of the TB bacteria with the antibiogram. After collecting participants' information, we diagnosed tuberculosis by detecting acid-fast bacilli (AFB) in sputum using the Ziehl-Neelsen (ZN) method of acidfast staining technique. It was then processed with TB bacteria cultured on MGIT consisting of a liquid broth medium using the BACTEC MGIT960 (320) automated¹⁰.

After the bacterial colonization had occurred, we identified Mycobacteria by Catalase test and used the Niacin test to differentiate the bacteria¹⁰. Then, we cultured TB bacteria on Lowenstein Jensen medium (L J), and an antibiogram was also conducted on the bacteria. Preparation of the growth medium and control strains were essential steps. The antibiogram was tested for quality in batch culture by controlling strains with 37HRV strain, resistant to HE strain, and resistant to RS strain. This was followed by making a bacterial suspension and culturing it into the medium¹⁰.

The following formula was used to calculate the resistance rate:

% Antibiotic resistance rate = [(Number of colonies counted in L J medium containing antibiotic/Number of colonies counted in L J medium without antibiotic) \times 100]

2.5. Method for identifying rifampicin-resistant gene mutations in MDR-TB

To extract DNA from TB bacteria, we performed the same steps as the study on drug-resistant TB, from culture on liquid medium to performing antibiogram. After that, the bacterial DNA from the liquid culture medium was obtained and extracted with reagent kits by Sacace company (Italy).

The highly purified samples after the extraction were used as a DNA template for PCR reaction with 411bp length to amplify the *rpoB* gene fragments with a specific primer pair.

• rpoB-F: 5' TAC GGT CGG CGA GCT GAT CC 3'

• rpoB-R: 5' TAC GGC GTT TCG ATG AAC C 3'

The PCR mix components and thermal cycles were set up following Yuen L. K. W. et al.'s studies method¹¹. We conducted the processes at the molecular biology department of the Institute of Biotechnology Research and Development, Can Tho University. Tuberculosis DNA samples after extraction were measured for concentration and purity while used subsequently as a template to amplify the *rpoB* gene region through specific primers. We conducted the processes by using a program consisting of an initial denaturation at 94°C for 3 min, followed by 25 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 60°C, and extension for 1 min at 72°C and an additional extension step at 72°C for 5 min. PCR products were determined by electrophoresis on 1% agarose agar. Ultimately, the results were obtained after sequencing by the company Macrogen.

2.6. Analysis and statistics

2.6.1. Determining the rate of antituberculosis drugs resistance

The obtained data were analyzed using the statistical program SPSS 18.0, including descriptive statistical methods and the chi-square test.

2.6.2. Determining the rate of antituberculosis drugs resistance

Using Mega 5.0 and BioEdit 7.0.5.3 software, obtained data were processed and compared with the GeneBank database (accession no. L27989)¹¹.

2.7. Ethics approval

With the patients' consent, participants in the trial were instructed to take sputum for testing without affecting their health. We believe that this study did not violate medical ethical considerations based on the study's advantages and safe implementation. The approved code was 4087/QD-DHCT.

3. RESULTS

3.1. Sample selection process

3.1.1. Study on the rate of antibiotic resistance rate in recurrent pulmonary TB

Two hundred forty-six patients were diagnosed with recurrent pulmonary tuberculosis and were given an antibiotic sensitivity test. The results showed that there were 157 patients with drug-resistant pulmonary tuberculosis. There were 57 individuals with multidrugresistant pulmonary tuberculosis among them (MDR-TB).

3.1.2. Study on the mutation gene of resistance to rifampicin in MDR-TB

The sputum samples were cultured and identified as tuberculosis bacteria. There were 40 strains of TB bacteria with results of first-line antituberculosis drugs (resistant to rifampicin and isoniazid). Our findings revealed that 30 strains have mutations in the rifampicin resistance determining region (RRDR). Ten strains of TB bacteria with antibiogram results exhibited MDR/ RR-TB; however, sequencing data for the 81bp region of the *rpoB* gene did not detect any mutations. The remaining two were sensitive to rifampicin, similar to the original strain from the NCBI GeneBank database (accession no. L27989).

3.2. Patient characteristics in the study

Regarding the characteristics of participating patients, the proportion of male patients was approxi-

mately four times that of female patients. The group with the most significant number of participants was 30 to 60 years old, while the under 30 years old group had the fewest patients. Table 1 summarizes the participants' characteristics.

3.3. Percentages of single-drug and multidrug resistance in recurrent pulmonary tuberculosis

We created the antibiogram after culturing the samples in Lowenstein-Jensen medium. According to the findings, the overall rate of drug-resistant TB bacteria was 63.8 percent, and MDR-TB accounted for 23.2 percent of cases. The percentages of TB bacteria that were drug-susceptible and resistant to antituberculosis drugs were depicted in Table 2 and Table 3.

| Fable 1. Gender and age | group characteristics | of the participants in the study. |
|-------------------------|-----------------------|-----------------------------------|
|-------------------------|-----------------------|-----------------------------------|

| | Characteristics | Total patients (n=246) |
|----------------------|-----------------|------------------------|
| Gender | Male | 195 (79.3%) |
| | Female | 51 (20.7%) |
| | <30 | 25 (10.2%) |
| Age range (in years) | 30-60 | 170 (69.1%) |
| | >60 | 51 (20.7%) |

Table 2. The rates of single-drug resistance in the study.

| Isoniazid (n=246) | | Rifampicin (n=246) | | Ethambutol | | Strept | omycin 246) | Pyrazinamide | | |
|----------------------|-------|-----------------------|------|------------|-------|--------|----------------|--------------|------|--|
| N | % | N (III | % | N | % | N | % | N | % | |
| 116 | 47.15 | 62 | 25.2 | 29 | 11.79 | 129 | 52.44 | 24 | 9.76 | |

Table 3. The rates of multidrug resistance in the study.

| Sensitive | N (n=246) | 89 |
|------------------------|-----------|-------|
| | % | 36.19 |
| Single-drug resistance | N (n=246) | 53 |
| | % | 21.54 |
| 2 drugs resistance | N (n=246) | 42 |
| | % | 17.07 |
| 3 drugs resistance | N (n=246) | 33 |
| | % | 13.41 |
| 4 drugs resistance | N (n=246) | 21 |
| | % | 8.54 |
| 5 drugs resistance | N (n=246) | 8 |
| | % | 3.25 |

3.4. Detection of the rifampicin-resistant mutated gene in MDR-TB

Mutations on the RRDR (81bp) of the *rpoB* gene were detected to determine the rifampicin-resistant mutant genotypes and their prevalence. Figure 1 illustrates the position of the PCR product (411bp) after gel electrophoresis. We used the software Mega 5.0 and BioEdit 7.0.5.3 to analyze and compare with NCBI gene bank. The red frame in Figure 2 indicated the location of a

substitution mutation that could alter the binding of rifampicin in the active site of RNA polymerase and lead to antibiotic resistance. The 4-colored peaks represented the nucleotides. After analysis, we found that up to 75% of mutations occurred in the RRDR, including 13 codons with substitution mutations. The above results are described in Table 4. The remaining strains had rifampicin resistance results from the antibiogram; however, we did not find mutations occurring in the RRDR of the *rpoB* gene.

| Codon | Codon change | Amino acid substitution | Quant | ity of strains |
|-------|-----------------------|---------------------------------------|-------|----------------|
| | (wild type -> mutant) | | | |
| 507 | $GGC \rightarrow GAC$ | Glycine \rightarrow Aspartic acid | 1 | (2.5%) |
| 510 | $CAG \rightarrow GAG$ | Glutamine \rightarrow Glutamic acid | 3 | (7.5%) |
| 511 | $CTG \rightarrow GTG$ | Leucine \rightarrow Valine | 2 | (5%) |
| 513 | $CAA \rightarrow CTG$ | Glutamine \rightarrow Leucine | 1 | (2.5%) |
| | \rightarrow TTG | Glutamine \rightarrow Leucine | 1 | (2.5%) |
| 516 | $GAC \rightarrow GTC$ | Aspartic acid \rightarrow Valine | 2 | (5%) |
| | \rightarrow GCC | Aspartic acid \rightarrow Alanine | 2 | (5%) |
| 517 | $CAG \rightarrow AAG$ | Glutamine \rightarrow Glycine | 1 | (2.5%) |
| 518 | $AAC \rightarrow ACC$ | Asparagine \rightarrow Threonine | 1 | (2.5%) |
| 519 | $AAC \rightarrow AGC$ | Asparagine \rightarrow Serine | 1 | (2.5%) |
| 522 | $TCG \rightarrow GCC$ | Serine \rightarrow Alanine | 1 | (2.5%) |
| 523 | $GGG \rightarrow GTG$ | Glycine \rightarrow Valine | 7 | (17.5%) |
| 531 | $TCG \rightarrow TTG$ | Serine \rightarrow Leucine | 8 | (20%) |
| | \rightarrow TGT | Serine \rightarrow Cysteine | 2 | (5%) |
| | \rightarrow AAC | Serine \rightarrow Asparagine | 1 | (2.5%) |
| 532 | $GCG \rightarrow CCG$ | Alanine \rightarrow Proline | 1 | (2.5%) |
| 533 | $CTG \rightarrow CGG$ | Leucine \rightarrow Arginine | 2 | (5%) |
| | \rightarrow CCG | Leucine \rightarrow Proline | 3 | (7.5%) |
| | \rightarrow ACG | Leucine \rightarrow Threonine | 1 | (2.5%) |

Table 4. Substitution mutations with amino acid changes in the RRDR of the rpoB gene.



Figure 1. The PCR product length is 411bp in an agarose gel.



Figure 2. The nucleotide peaks on the sequence diagram.

4. DISCUSSION

The overall antituberculosis drug resistance in Can Tho was 63.8% at the end of the study. When we compared our studies to those of others, we discovered differences in the total rate of drug resistance among patients with recurrent pulmonary TB between countries and provinces within countries¹²⁻¹⁸. Table 5 represents the above distinction. The two places with the highest antibiotic-resistant rates were Baku, Azerbaijan, and Tashkent, Uzbekistan¹⁷⁻¹⁸. They were higher than those in Can Tho; however, our study's results are consistent with the percentage of the south of Vietnam¹³ and Hai Phong¹². In general, Vietnam was in areas with middlehigh rates of resistance.

Meanwhile, our study's multidrug-resistant pulmonary tuberculosis (MDR/RR-TB) rate was 23.17% (CI: 95%: 18.3-28.9). Comparison with other places also shows a variation in this ratio between different regions. The percentages are described in Table 6. According to the national tuberculosis program data, they had been published through 4 surveys, the results of multi-resistant TB in Vietnam were 32.5%, respectively; 23.5%, 19%, and 17.1%¹⁹. These results indicated that multidrugresistant TB in Vietnam is gradually decreasing. However, our findings showed that the rate of multidrugresistant pulmonary tuberculosis was 23.2%. When compared to the studies in Hai Phong (2011)¹², Thailand $(2009)^{20}$, and Iran $(2011)^{14}$, our results are higher. According to WHO (Global Tuberculosis Report 2014-2020), the rate of MDR/RR in recurrent cases in Vietnam in the 2013-2014 period was 23%, consistent with our results of 23.17 %. In the following years, the aforementioned rate was 25% (2015), 26% (2016), and 17% (2017-2019)²¹.

| Country/Region | Isoniazid | Rifampicin | Ethambutol | Streptomycin | Pyrazinamide | Overall |
|--|--------------------|------------|------------|--------------|--------------|---------|
| | (%) | (%) | (%) | (%) | (%) | (%) |
| The south of Vietnam (2006) ¹³ | 16.6 | 2 | 1.1 | 19.4 | NR | 62.9 |
| Baku, Azerbaijan (2009) ¹⁵ | Not Report (NR) | NR | NR | NR | NR | 84.4 |
| Tashkent, Uzbekistan (2009) ¹⁵ | NR | NR | NR | NR | NR | 85.9 |
| Shanghai, China (2009) ¹⁶ | 19.6 | 13.4 | 3.1 | 17.9 | NR | 27.9 |
| Hai Phong, Vietnam (2011) ¹² | 76.8 | 75.4 | 5.8 | 11.6 | NR | 53.9 |
| Iran (2011) ¹⁴ | 45.3 | 36.2 | 30.3 | 40.6 | 24.4 | 69.0 |
| Can Tho (our study, 2014) | 47.15 | 25.2 | 11.79 | 52.44 | 9.76 | 63.82 |

Table 5. Comparison of the prevalence of drug-resistant pulmonary TB in studies by country and region.

Table 6. Comparison of the percentage of MDR-TB in studies by country and region.

| Country or region | Resistance rate (%) |
|---|---------------------|
| Vietnam (2004-2005) ¹⁸ | 19.0 |
| Baku, Azerbaijan (2008) ¹⁷ | 55.8 |
| Tashkent, Uzbekistan (2008) ¹⁷ | 60.0 |
| Thailand (2009) ¹⁹ | 34.5 |
| Shanghai, China (2009) ¹⁶ | 9.7 |
| Hai Phong, Vietnam (2011) ¹² | 33.6 |
| Iran (2011) ¹⁴ | 31.7 |
| Can Tho (our study, 2014) | 23.2 |

What do the findings with the MDR/RR-TB ratio rise from 2013 to 2016? This question suggests further research.

Table 7 displays the mutation and rate on the codons of the study. The results showed that substitutions at two codons were similar to previous studies with different frequencies²²⁻²³. Furthermore, the mutations at three codons were consistent with previous studies^{24,22}. However, our find revealed a mutation in 7 codons (512-513-518-520-522-531-532), with hitherto unseen results.

Table 8 compares the difference in mutant genotypes of rifampicin-resistant TB bacteria in our study to studies from other geographical regions. Codon 526 is one of the most prevalent mutations in the RRDR of the rpoB gene²⁵⁻²⁸. Mutations at codon 526 had been found in previous studies^{23,25,27,29-30}. In comparison, our study in Can Tho found no substitution in this codon. However, our study detected a substitution mutation at codon 532 that causes rifampicin resistance, while prior studies had not found it^{22-25,27,29-35}. Fitzgibbon, Margaret M., et al. 2021 revealed mutations in Condon 532²⁶; however, the study's conclusion was due to the detection error of Xpert[®] MTB/RIF Ultra. Generally, these findings may prove that the mutation detected in our study is specific to Can Tho city. In addition, the substitution mutation had not been documented in the preceding reports. These results confirm that the rate of drug resistance and drug-resistant mutant strains of TB bacteria need to be researched in numerous areas.

Moreover, a history of smoking is an independent risk factor for antitubercular drug resistance³⁶, and diabetes can significantly increase the development of MDR-TB³⁷, particularly in elderly patients. Our study has not yet wholly managed the patients' lifestyle and history of background disease. These are drawbacks of our study, but we overcame them by using patients' history surveys to help better manage and evaluate their information. Furthermore, mutations in ponA1 and rpoC genes also cause resistance to rifampicin in TB bacteria³⁸. Because of the subject-matter limitations, this study was not conducted to sequence those genes. We attempted to overcome this restriction by thoroughly sequencing the *rpoB* gene and comparing it to prior studies. Likewise, the sequencing assay demonstrated high sensitivity for detecting RIF (96.92%), and the assay's specificities were 98.35% for RIF³⁹. This substantiation increased the reliability of the study.

In general, the rate of drug resistance in Can Tho is in the medium-high group. Our study provides information for clinicians in considering using antibiograms for drug-resistant TB patients to increase treatment efficiency and save costs. GeneXpert is a rapid diagnostic test for pulmonary tuberculosis; however, some mutant strains will not be detected because of false negatives. Our study contributes to data on mutated TB bacteria, thereby helping to diagnose earlier. In addition, early detection of drug-resistant TB will help clinicians create a plan and change drug-resistant TB treatment regimens

 Table 7. The percentage of mutations and the number of mutated rpoB codons.

| Number of codons | Mutated rpoB codon | Codon change Amino acid substitution | | Mutated codons |
|------------------|--------------------|--------------------------------------|---|----------------|
| | | (wild type -> mutant) | | (%) |
| 1 | 507 | $GGC \rightarrow GAC$ | $Gly \rightarrow Asp$ | 1 (2.5%) |
| | 512 | $AGC \rightarrow TCC$ | $\text{Ser} \rightarrow \text{Ser}$ | 1 (2.5%) |
| | 516 | $GAC \rightarrow GTC$ | $Asp \rightarrow Val$ | 1 (2.5%) |
| | 523 | $GGG \rightarrow GTG$ | $Gly \rightarrow Val$ | 1 (2.5%) |
| | 531 | $TCG \rightarrow TTG$ | $\text{Ser} \rightarrow \text{Leu}$ | 6 (15%) |
| | 531 | $TCG \rightarrow TGT$ | $\text{Ser} \rightarrow \text{Cys}$ | 2 (5%) |
| | 532 | $GCG \rightarrow CCG$ | $Ala \rightarrow Pro$ | 1 (2.5%) |
| | 533 | $CTG \rightarrow CGG$ | $Leu \rightarrow Arg$ | 1 (2.5%) |
| | 533 | $CTG \rightarrow CCG$ | $Leu \rightarrow Pro$ | 1 (2.5%) |
| 2 | 516 and 533 | $GAC \rightarrow GTC$ | $Asp \rightarrow Val$ | 1 (2.5%) |
| | | $CTG \rightarrow CGG$ | $Leu \rightarrow Arg$ | |
| | 512 and 513 | $AGC \rightarrow TCG$ | $\text{Ser} \rightarrow \text{Ser}$ | 1 (2.5%) |
| | | $CAA \rightarrow CAG$ | $Gln \rightarrow Gln$ | |
| | 531 and 533 | $TCG \rightarrow TTG$ | $\text{Ser} \rightarrow \text{Leu}$ | 1 (2.5%) |
| | | $CTG \rightarrow CCG$ | Leu →Pro | |
| | 513 and 523 | $CAA \rightarrow CAG$ | $Gln \rightarrow Gln$ | 1 (2.5%) |
| | | $GGG \rightarrow GTG$ | $Gly \rightarrow Val$ | |
| 3 | 510-511-523 | $CAG \rightarrow GAG$ | $Gln \rightarrow Glu$ | 1 (2.5%) |
| | | $CTG \rightarrow GTG$ and CTC | Leu \rightarrow Val, \rightarrow Leu | |
| | | $CGG \rightarrow GTG$ | $Gly \rightarrow Val$ | |
| | 511-512-513 | $CTG \rightarrow GTG$ and CTC | Leu \rightarrow Val and Leu | 1 (2.5%) |
| | | $AGC \rightarrow TCG$ | $\text{Ser} \rightarrow \text{Ser}$ | |
| | | $CAA \rightarrow CAG$ | $Gln \rightarrow Gln$ | |
| | 510-513-523 | $CAG \rightarrow GAG$ | $Gln \rightarrow Glu$ | 1 (2.5%) |
| | | $CAA \rightarrow CTG$ | $Glu \rightarrow Leu$ | |
| | | $GGG \rightarrow GTG$ | $Gly \rightarrow Val$ | |
| | 513-516-533 | $CAA \rightarrow CAG$ | $\operatorname{Gln} \to \operatorname{Gln}$ | 1 (2.5%) |
| | | $GAC \rightarrow GCC$ | $Asp \rightarrow Ala$ | |
| | | $CTG \rightarrow CCG$ | $Leu \rightarrow Pro$ | |
| | 513-516-523 | $CAA \rightarrow CAG$ | $\operatorname{Gln} \to \operatorname{Gln}$ | 1 (2.5%) |
| | | $GAC \rightarrow GCC$ | $Asp \rightarrow Ala$ | |
| | | $GGG \rightarrow GTG$ | $Gly \rightarrow Val$ | |
| 4 | 510-523-531-533 | $CAG \rightarrow GAG$ | $\operatorname{Gln} \rightarrow \operatorname{Glu}$ | 1 (2.5%) |
| | | $GGG \rightarrow GTG$ | $Gly \rightarrow Val$ | |
| | | $TCG \rightarrow TTG$ | $\text{Ser} \rightarrow \text{Leu}$ | |
| | | CTG →ACG | $\text{Leu} \rightarrow \text{Thr}$ | |
| | 512-513-519-523 | $AGC \rightarrow TCG$ | $\text{Ser} \rightarrow \text{Ser}$ | 1 (2.5%) |
| | | $CAA \rightarrow CAG$ | $\operatorname{Gln} \to \operatorname{Gln}$ | |
| | | $AAC \rightarrow AAT$ | $Asn \rightarrow Asn$ | |
| | | $GGG \rightarrow GGT$ | $Gly \rightarrow Gly$ | |
| | 512-513-521-523 | AGC \rightarrow TCG and TCC | $\text{Ser} \rightarrow \text{Ser}$ | 2 (5%) |
| | | $CAA \rightarrow CAG$ | $Gln \rightarrow Gln$ | |
| | | $CTG \rightarrow TTG$ | $Leu \rightarrow Leu$ | |
| | | $GGG \rightarrow GGT$ | $Gly \rightarrow Gly$ | |
| | 513–517–519–523 | $CAA \rightarrow TTG$ | $Gln \rightarrow Leu$ | 1 (2.5%) |
| | | $CAG \rightarrow AAG$ | $Gln \rightarrow Gly$ | |
| | | $AAC \rightarrow AGC$ | $Asn \rightarrow Ser$ | |
| | | $GGG \rightarrow GTG$ | $Gly \rightarrow Val$ | |
| 7 | 512-513-518-520- | $AGC \rightarrow TCC$ | $\text{Ser} \rightarrow \text{Ser}$ | 1 (2.5%) |
| | 522-531-532 | $CAA \rightarrow CAG$ | $Gln \rightarrow Gln$ | |
| | | $AAC \rightarrow ACC$ | $Asn \rightarrow Thr$ | |
| | | $CCG \rightarrow CCC$ | $Pro \rightarrow Pro$ | |
| | | $TCG \rightarrow GCC$ | $\text{Ser} \rightarrow \text{Ala}$ | |
| | | $TCG \rightarrow AAC$ | $\text{Ser} \rightarrow \text{Asn}$ | |
| | | $GCG \rightarrow GCC$ | $Ala \rightarrow Ala$ | |

Table 8. Differences between the mutated codons of the rpoB gene in the study of MDR-TB by country and region.

| Can Tho (n=40) | 507 | 510 | 511 | 513 | 516 | 517 | 518 | 519 | 522 | 523 | 531 | 532 | 533 |
|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Singapore (n=51; 2005) ²² | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 0 | + | 0 | 0 |
| Vietnam (n=104; 2006) ³¹ | 0 | 0 | + | + | + | 0 | 0 | 0 | 0 | 0 | + | 0 | + |
| Belarus (n=44; 2006) ²⁹ | + | + | 0 | 0 | + | 0 | 0 | 0 | 0 | + | + | 0 | 0 |
| Turkey (n=19; 2008) ³⁰ | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 |
| Iran (n=46; 2009) ²³ | 0 | 0 | + | + | 0 | del | del | 0 | + | 0 | + | 0 | 0 |
| Guangdong, China (n=160; 2009) ³⁴ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | 0 | + |
| Guizhou, China (n=32; 2010) ²¹ | 0 | 0 | + | 0 | + | 0 | 0 | 0 | + | 0 | + | 0 | + |
| India (n=50; 2010) ³³ | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 |
| Vietnam (n=74; 2012) ³² | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | + | 0 | + | 0 | + |
| Kyrgyz Republic (n=157; 2018) ²⁶ | 0 | 0 | + | + | + | 0 | 0 | 0 | + | 0 | + | 0 | + |
| Zambia (n=99; 2020) ²⁸ | 0 | 0 | + | + | + | 0 | + | 0 | + | 0 | + | 0 | + |
| Thailand (n=30: 2020) ²⁴ | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | + | 0 | + | 0 | 0 |

earlier for patients; thus, they can save costs and time of medical treatments. The mutation in codon 531 accounted for the highest percentage, consistent with the studies^{25,27,29}. These results confirm the importance of codon 531 in the rifampicin resistance mutant. Additionally, the promising results of this study imply that further studies on this issue with larger sample sizes and more antibiotics are needed to acquire a comprehensive picture of mutations associated with resistance to antituberculosis drugs in Can Tho. Our findings also pave the path for further studies on rifampicin resistance mutations in other genes and research on factors affecting antibiotic resistance and gene mutations.

5. CONCLUSION

In conclusion, our study provided the rate of firstline antituberculosis drugs in recurrent tuberculosis and rifampicin-resistant mutations in RR/MDR-TB. The encouraging results of this study showed that we detected a new substitution mutation on codon 532 of the *rpoB* gene and a mutant strain of tuberculosis bacteria at all seven codons of this gene. The study provided information on drug resistance in Can Tho, Vietnam, and contributed to previous studies explaining MDR-TB. Further studies on the clinical application for effective TB treatment and control are suggested.

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Conflict of interest

The authors have stated no competing interests in this study and publication.

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Ethics approval

With the patients' consent, participants in the trial were instructed to take sputum for testing without affecting their health. We believe that this study did not violate medical ethical considerations based on the study's advantages and safe implementation. The approved code was 4087/QD-DHCT.

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