

Research Article

RESEARCH ON *rpoB* GENE MUTATIONS OF *Mycobacterium tuberculosis* RESISTANT TO RIFAMPICIN IN VINH LONG LUNG HOSPITAL

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ABSTRACT

Introduction: Rifampicin resistance due to infection with *Mycobacterium tuberculosis* can be mutated on many typical genes and some related genes, especially *rpoB* gene of tuberculosis patients treated at Vinh Long lung hospital with molecular biotechnological techniques with high-precision to identify mutations in resistance genes to help doctors treat patients effectively. **Research objectives:** (1) Determine the rate of rifampicin resistance by *rpoB* gene sequencing on *Mycobacterium tuberculosis* from patients treated in the hospital and some related factors in tuberculosis patients at Vinh Long Lung Hospital. (2) Determine the rate and type of mutations in *rpoB* gene in tuberculosis patients resistant to rifampicin. **Subjects and research methods:** A cross-sectional descriptive study on 412 patients with GeneXpert test results with TB bacteria at Vinh Long Lung Hospital. **Results:** Rifampicin resistance rate by Gene Xpert technique in patients with tuberculosis is 4.6%. The relapsed TB group has a higher rate of rifampicin resistance compared with the new TB group ($p < 0.001$). The group of civil servants has a rate of rifampicin resistance 31 times higher than that of the farmer group with $p = 0.019$. Gene sequence analysis of 14 cases of rifampicin resistance shows that there are 2 mutated codon positions, 531 and 526, in which codon position 531 is mutated at the S531L position of Ser → Leu with 08 samples accounting for 57.1% of mutations, at the H526N position, His → Asn has 02 samples, accounting for 14.3%, the H526L position changes from His → Leu has 02 samples, accounting for 14.3%, the H526D position changes from His → Asp has 01 sample accounting for 7.1% and the position of H526Y changes from His → Tyr has 01 sample, accounting for 7.1%. Mutation sites on the *rpoB* gene are found in codon 526 and codon 531. **Conclusion:** The rate of Rifampicin resistance at Vinh Long Lung Hospital is quite high. There is a relation between Rifampicin resistance with prehistory of tuberculosis treatment ($p < 0.001$) and occupation ($p < 0.019$). Mutation sites of the *rpoB* gene are found at codons 526 and 531, respectively.

Keywords: *rpoB* gene mutation, multidrug resistance, Tuberculosis, antibiotics, rifampicin resistance.

INTRODUCTION

Tuberculosis is now a serious social disease due to its rapid spread in the community according to a report from the World Health Organization. Despite some remarkable achievements in the fight against tuberculosis in recent years, Tuberculosis continues to be one of the major public health problems globally. The World Health Organization estimates that in 2019 there was about 10 million people with tuberculosis in the world, a number that has been declining very slowly in recent years; 8.2% of TB patients had HIV co-infection. Tuberculosis is the second leading cause of death among infectious diseases with about 1.2 million deaths from TB and about 208,000 deaths from TB (with HIV). This number of deaths makes tuberculosis one of the world's leading causes of death. Globally, in 2019 the prevalence of MDR-TB was estimated at 3.3% among new patients and 17.7% in re-treated patients [1]. Among the drugs for tuberculosis treatment, rifampicin is the most interested, but due to mutations in the genotype of TB bacteria, resistance to rifampicin leads to failure in TB treatment today. According to the WHO's definition, "multi-drug resistant TB" is the least resistance to rifampicin and another drug [2]. Anti-TB drug resistance can be mutated in many genes, but typically a few genes are involved, especially the *rpoB* gene. Studies on drug resistance of TB bacteria show that very few strains are resistant to rifampicin alone, but most of strains resistant to rifampicin are also resistant to isoniazid, also known as multidrug-resistant [26].

Until now, the detection of drug-resistant tuberculosis has mainly relied on bacterial culture to make antibiotic charts, which usually takes about 4-8 weeks to get the results. Today, with the great development of science and technology, especially in the field of medicine such as the Xpert gene molecular biology test, it helps to quickly diagnose patients infected with drug-resistant TB bacteria within just two hours for results and sequencing method. Therefore, the application of molecular biology techniques is a highly accurate job to identify mutations in the nucleotides of a drug resistance gene to help doctors use highly effective drugs. From the above fact, we conducted the project "Research on *rpoB* gene mutation of *Mycobacterium tuberculosis* resistant to Rifampicin in Vinh Long Lung Hospital in 2022-2023"

Objectives of the research:

1. Determine rifampicin resistance rate by Gene Xpert technique and some related factors in tuberculosis patients in Vinh Long Lung Hospital.
2. Determine the rate and type of mutations in *rpoB* gene in patients with tuberculosis resistant to rifampicin.

II. RESEARCH SUBJECTS AND METHODS

2.1. Subjects

2.1.1. Research subjects

Tuberculosis patients came to Vinh Long Lung Hospital from June 2022 to April 2023

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2.1.2. Sample selection criteria

- Patients with TB bacteria in their GeneXpert test results
- 18 years old and older
- Consent to participate in the study.

2.1.3. Exclusion criteria

- Patients in severe condition cannot walk on their own and cannot provide qualified sputum samples.

2.1.4. Place and time of research

- Place of research: Vinh Long Lung Hospital.
- Time of research: from July 2022 to April 2023.

2.2. Research method

2.2.1. Research Design: A cross-sectional descriptive research.

2.2.2. Sample size:

Apply the formula to calculate the estimated sample size of 1 ratio:

$$n = Z^2_{(1-\frac{\alpha}{2})} \frac{p(1-p)}{d^2}$$

In which:

n: the estimated sample for the research.

p: Author Hoang Ha noted that the rate of resistance to rifampicin in Thai Nguyen from 2020 is 4%.

d: the absolute error, choose d = 0.02.

α: Select the 95% statistical level, with α=0.05.

Z(1- α/2): The value obtained by looking up the Z table, α = 0.05; Z = 1.96. Substituting into the formula, get n = 369

Expect 10% for sample loss rate

Therefore, the estimated sample size for the research is: 369+ 36.9 ≈ 406 samples, actually taking 412 samples in the research.

2.2.3. Sample selection method

Convenience sampling: All patients who met the sampling criteria were selected for the study until the estimated sample size was sufficient.

2.3. Identification of mutations in the rpoB gene

+ The sequence of rpoB genes of tuberculosis bacteria is looked up on the International Gene Bank (Genbank)

+ For rpoB gene, use primer pair

rpoB-F 5'-ACC GAC GAC ATC GAC CAC TT-3'

rpoB-R 5'- GGC GGT CAG GTA CAC GAT CT-3'

2.4. Data processing and analysis methods

- Use SPSS 20.0 software for statistical analysis. Collected data are coded and cleaned before being processed by SPSS 20.0 software.
- Analysis of sequencing results by Bio Edit software.

III. RESEARCH RESULTS

In fact, we obtain 412 samples of subject information, conduct a survey and record the following results:

3.1. Ratio of men and women in the study

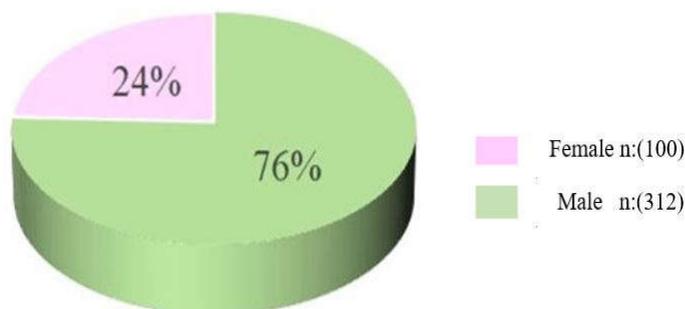


Figure 3.1. Distribution of research subjects by gender

Note: The majority of research subjects are male, accounting for 75.7%, 3 times higher than female (24.3%).

3.2. Distribution by age group in the research

Table 3.1. Distribution of patients according to age group

| Age group | Quantity (n) | Ratio (%) |
|-----------------|--------------|-----------|
| 18-29 years old | 19 | 4,6 |
| 30-45 years old | 89 | 21,6 |
| 46-59 years old | 142 | 34,5 |
| ≥60 years old | 162 | 39,3 |
| Total | 412 | 100 |
| Average age | 55,47±30,62 | |

Note: The average age of the research group is 55.47±30.62 years old, the youngest age is 18 years old, the highest age is 94 years old. The age group ≥60 years old accounts for the highest rate of 39.3%, followed by the age group 46-59 with 34.5%, the age group 30-45 and 18-29 are 21.6% and 4.6 % respectively.

3.3. Distribution by educational level in the research

Table 3.2. Distribution of patients by educational level

| Educational level | Quantity(n) | Ratio (%) |
|---|-------------|-----------|
| Illiterate | 64 | 15,5 |
| Primary school (grade 1-5) | 229 | 55,6 |
| Secondary school (grade 6-9) | 74 | 18 |
| High school (grade 10-12) | 42 | 10,2 |
| Technical school/college/university/postgraduate university | 3 | 0,7 |
| Total | 412 | 100 |

Note: Most of the patients have primary education (55.6%), secondary school education (18%), high school education (10.2%), above high school education (0.7%), illiteracy (15.5%)

3.4. Distribution by occupation in the research

Table 3.3. Distribution by occupation

| Jobs | Quantity (n) | Ratio (%) |
|----------------|--------------|-----------|
| Farmers | 195 | 47,3 |
| Workers | 74 | 18 |
| Civil servants | 2 | 0,5 |
| Traders | 66 | 16 |
| Students | 4 | 1,0 |

| | | |
|--------------|------------|------------|
| Others | 71 | 17,2 |
| Total | 412 | 100 |

Note: Farmer occupation accounts for the majority (47.3%), followed by workers (18%) and traders (16%), students (1%), civil servants (0.5 %), other occupations (17.2%).

3.5. Analysis by hematological and biochemical values of patients infected with Mycobacterium tuberculosis in the research

Table 3.4. Red blood cells and white blood cells indicators

| Indicator | | Average value (cell/mm ³) | Smallest value (cell/mm ³) | Biggest value (cell/mm ³) |
|------------|------------------|---------------------------------------|--|---------------------------------------|
| Hematology | Red blood cell | 4,4±0,8 | 1,5 | 7,7 |
| | White blood cell | 10,4±3,8 | 3,5 | 30,9 |

Note:

- The average value of red blood cells in patients infected with Mycobacterium tuberculosis in the study is: 4.4±0.8 cells/mm³ and the average value of white blood cells is: 10.4±3, 8 cells/mm³. It shows that it is suitable for Mycobacterium tuberculosis infection.

Table 3.5. White blood cell centigrade formula

| Indicator | | Average value (cell/mm ³) | Smallest value (cell/mm ³) | Biggest value (cell/mm ³) |
|-------------------------------------|-----------------|---------------------------------------|--|---------------------------------------|
| White blood cell centigrade formula | Neutrophile (%) | 71,5±14,1 | 7,1 | 95,2 |
| | Lymphocyte (%) | 19,3±11,8 | 1,1 | 75,5 |

Note:

- The average value of neutrophils (%) is: 71.5±14.1 cells/mm³ and the average value of lymphocytes (%) is: 19.3±11.8 cells/mm³. It shows that this is also appropriate in Mycobacterium tuberculosis infection.

Table 3.6. Biochemical values

| Indicator | | Average value | Smallest value | Biggest value |
|-----------------|---------------------|---------------|----------------|---------------|
| Liver function | SGOT (U/L) | 61,4±314,7 | 11 | 6405 |
| | SGPT (U/L) | 47,9±70,7 | 10 | 1295 |
| Kidney function | Ure (µmol/L) | 4,9±2,5 | 2,3 | 32 |
| | Creatinine (µmol/L) | 76,2±17,1 | 23 | 160 |

Note:

- The average SGOT index is 61.4±314.7, the highest value of this index is 6405U/L, the lowest is 11U/L
- The average SGPT index is 47.9±70.7, the highest value is 1295U/L, the smallest is 10U/L.
- The average Urea index is 4.9±2.5, the highest value is 32 µmol/L, the lowest is 2.3 µmol/L.
- The average Creatinine index is 76.2±17.1, the highest value is 160µmol/L, the lowest is 23µmol/L.

3.6. Analysis according to lesion characteristics on X-ray film of patients infected with Mycobacterium tuberculosis in the study

Table 3.7. Characteristics of lesions on X-ray film

| Xray injury | Quantity (n) | Ratio (%) |
|---------------------|--------------|------------|
| Tuberculosis cave | 44 | 10,7 |
| 1- sided infiltrate | 119 | 28,9 |
| 2- sided infiltrate | 249 | 60,4 |
| Total | 412 | 100 |

Note: Most of the patients with lesions on X-ray film are infiltrates in both lungs, accounting for 60.4%, infiltrates in 1 disease accounts for 28.9% and only 10.7% has images of tuberculosis cave on X-ray film.

3.7. Rate of rpoB gene mutation in rifampicin-resistant Mycobacterium tuberculosis in Vinh Long Lung Hospital

The results of the study of 14 patient samples shows that the rpoB gene mutation of Mycobacterium tuberculosis resistant to rifampicin is shown in the table below.

Table 3.8. Rifampicin - resistant rpoB gene mutation positions

| Codon position | Mutation position | Acid amin | Quantity | Ratio |
|----------------|-------------------|-----------|-----------|------------|
| 511 | L511P | Leu→Pro | 0 | 0 |
| 512 | S512G | Ser→Gly | 0 | 0 |
| 513 | Q513K | Gln→Lys | 0 | 0 |
| | Q513L | Gln→ Leu | 0 | 0 |
| 516 | D516G | Asp→Gly | 0 | 0 |
| | D516A | Asp→ Ala | 0 | 0 |
| | D516V | Asp→Val | 0 | 0 |
| 526 | H526N | His→ Asn | 2 | 14,3 |
| | H526D | His→ Asp | 1 | 7,1 |
| | H526Q | His→ Gln | 0 | 0 |
| | H526S | His→ Ser | 0 | 0 |
| | H526R | His→Arg | 0 | 0 |
| | H526L | His→ Leu | 2 | 14,3 |
| 531 | H526Y | His→ Tyr | 1 | 7,1 |
| | H526F | His→ Phe | 0 | 0 |
| 531 | S531L | Ser→Leu | 8 | 57,1 |
| 533 | L533P | Leu→Pro | 0 | 0 |
| 561 | I561V | Ile→Val | 0 | 0 |
| Total | | | 14 | 100 |

Note: Analysis of gene sequences of 14 cases of rifampicin resistance shows that there are 2 mutated codon positions 531 and 526, in which codon position 531 is mutated at position S531L of (Ser → Leu) with 08 samples, accounting for 57,1% mutation. Position H526N (His → Asn) has 02 samples accounting for 14.3%, position H526L (His → Leu) has 02 samples accounting for 14.3%, position H526D (His → Asp) has 01 sample accounting for 7.1% and position H526Y (His → Tyr) has 01 sample accounting for 7.1%. All 14 patient samples are recorded that there is only Rifampicin resistance mutations at codon positions 526 and 531, the remaining codon positions do not record mutations, our study also did not record mutations at 2 or more positions of the rpoB gene.

IV. DISCUSSION

Distribution of patients according to age groups

All patients in our study are adults aged from 18 years. The number of isolated TB bacteria increases gradually by age group and accounts for the highest proportion in the elderly (≥ 60 years old) accounting for 39.3%, followed by the age group 46-59 accounting for 34.5%, age groups 30-45 and 18-29 are 21.6%, 4.6%, respectively. The average age is 55.47 ± 30.62 . The results are different with the author Mai Van Tuan, who notes that the age of TB is similar, respectively, 16-29 years old accounting for 21.5%, 30-45 years old accounting for 28.5%, 46-49 years old accounting for 22.4% and slightly increases in over 60 years old group, accounting for 26.7% [12]. Mohamed Muhumed Ali also records age characteristics in his research. For example, the average age of the study participants is 44.3 ± 14 years, which is lower than our results, and the age group 35-44 accounts for the majority (25.9%). There is a gradual decreasing in the age group 55-64 (18.4%) and the lowest is in the group over 64 years old (9.2%).

Distribution of patients by educational level and occupation

In our research, the majority of subjects (55.6%) has primary school education, 18% for those with secondary school education, 10.2% for those with high school education, 0.7% for those with higher education (higher than high school education), and 15.5% of research subjects are illiterate. Unlike author Nguyen Kim Cuong who makes the research in the Central Lung Hospital, Hanoi Lung Hospital, 9 out of 123 patients accounting for the highest rate of 45.5% have the high school education, 38.2% for the secondary school education, and 3.3% for the university education [5]. The occupation of farmers accounts for the majority (47.3%), followed by workers (18%) and traders (16%), students (1%), civil servants (0.5%), and other occupations (17.2%). Our research is similar to that of author Dao Thi Huong, this research notes that the percentage of patients working as farmers accounts for 71.7%, which is much higher than that of other occupations (28.3%). The results of our study are lower than the results of the author Hoang Ha (2021), which recorded that the majority of patients working as farmers accounted for 73.5% [7].

Agricultural occupation has a high rate of diseases because people working in agriculture often have low income, unsecured living standards, and they often face many difficulties in their daily lives. The lack of economic and material resources will affect the health as well as the people's ability to resist tuberculosis.

Clinical and subclinical features

The research shows that there are 4 main reasons for hospitalization of the research subjects, of which the highest and most common is fever (37.1%), followed by persistent cough (36.9%). Uncommon symptom is shortness of breath (19.2%) and symptoms of hemoptysis (6.8%). This result is lower than in the research of author Nguyen Kim Cuong, which find that there is a fever including fever of $38.6 \pm 2.4^\circ\text{C}$ (88.6%), persistent cough symptoms with the highest proportion (92.7%) followed by shortness of breath (28.5%) and the lowest one is hemoptysis accounting for 17.1% [5], but it is higher than that of the author Nguyen Huy Dien, this research records only 16.8% of patients hospitalized for fever, persistent cough accounts for the highest rate 69.2%, followed by hemoptysis 18.8% and shortness of breath with a relatively low rate of only 14.4% [6]. Routine hematology tests in patients with tuberculosis are of little diagnostic value, but they are of value in differentiating from infectious causes other than

tuberculosis or in assessing the patient's general condition. In this research, the average red blood cell count is 4.4 ± 0.8 , which is higher than that of the author Nguyen Kim Cuong, the average red blood cell count is 3.4 ± 0.9 (T/L), but equivalent to the results of Nguyen Thu Ha (2012), mean values of erythrocytes and hemoglobin in the multidrug resistant group (4.44 ± 0.77) are lower than those in the sensitive group (4.4 ± 1.02) and higher than those in the no multi-resistance group (4.19 ± 0.9) [8]. The average value of white blood cells is 10.4 ± 3.8 , in which the maximum value is 30.9. The average neutrophil rate is 71.5 ± 14 , the average lymphocyte rate is 19.3 ± 11.8 . This result is higher than Nguyen Kim Cuong's study on the average white blood cell count 6.4 ± 3.1 , the neutrophil rate $67.2 \pm 20\%$ but lower in the lymphocyte ratio $20, 4 \pm 14.4\%$ [5].

The average SGOT (AST) is 61.4 ± 314.7 , the highest value of this indicator is 6405. The average SGPT (ALT) is 47.9 ± 70.7 , the highest value is 1295. It is similar to the study of Nguyen Kim Cuong, the SGOT and SGPT indexes are both higher than normal levels, SGOT (AST) 61.8 ± 44 U/l, SGPT (ALT) 49.5 ± 37.4 respectively. This can be related to the side effects of some drugs, which can be a difficult factor for the patient's prognosis because anti-tuberculosis drugs are also drugs that have a risk of affecting the liver. The average Ure indicator is 4.9 ± 2.5 , the average Creatinine indicator is 76.2 ± 17.1 , this result is higher than that of Nguyen Kim Cuong in which the Ure indicator is $4,8 \pm 4,9$ and Creatinine indicator is 67.9 ± 20.8 [5]. In general, the indexes in this study were within normal limits. In this study, the majority of patients with lesions on X-ray film are infiltrated in both lungs, accounting for 60.4%, 1 sided infiltrating accounts for 28.9% and only 10, 7% has images of tuberculosis cave on X-ray film. This infiltrating rate is lower than that of some domestic researchs. For example, Nguyen Huy Dien's research with an infiltrating rate of 62.5%; Dang Vinh Hiep with heterogeneous infiltrating rate of 64.28%; and the highest rate in Nguyen Kim Cuong's research which is 68.3% [10], [6], [5].

Sequencing results to detect mutations in the rpoB gene

Detection rate of mutations on the rpoB gene

After sequencing 19 samples of Rifampicin-resistant TB bacteria (including MDR-TB multidrug-resistant samples), it is found that 14/19 samples with mutations in rpoB gene accounts for 73.7%, 5 samples are found any mutations. Our results are similar to many other studies in the country and around the world. The rate of these studies is much higher than that of Ngo Viet Quynh Tram, which finds 32 strains (80%) out of 40 strains of rifampicin resistant TB bacteria with rpoB gene mutations mainly in codon region 531 [21]. ; Ismail in the study is to detect genotypes of genrpoB and katG mutations related to Rifampicin and Isoniazid resistance in Mycobacterium tuberculosis strains recorded 80% of Ripampicin resistance mutations on rpoB [8]; Author Nghiem Ngoc Minh (2012) does not find any mutations in the rpoB gene of 10 sensitive strains of Ripampicin [12], ShahN. S. (2009), when sequencing 8 strains, the results of antibiotic resistance are incompatibility between the two methods INNO-LiPA and classical (sensitive to classical method but resistant to INNO-LiPA method), no mutation in the RRDR region of the rpoB gene [46] is found.

Rifampicin-resistant mutation sites

Sequence analysis of 14 cases of Rifampicin resistance shows that there are 2 mutated codon positions, 526 and 531, of which 57.1% of mutation is at the S531L position of codon 531 (Ser \rightarrow Leu). Mutations with 2 samples accounts for 14.3% mutations at the H526L position of codon 526 (His \rightarrow Leu). All 14 patient samples records only rifampicin resistance mutations in 1 codon position 526 or 531, the

remaining codon positions do not record mutations. Our study also does not record mutations in 2 or more positions of the rpoB gene. Alike Mai Van Tuan's research, this author also records 50% mutations on codon 531 (Ser → Leu), the mutations mostly focus at codon 531 (Ser→Leu), rate 32, 3%, in terms of mutations in codon 526, our results are higher than Mai Van Tuan's when we only obtain mutations at codon 526 with the rate of 12.9%. At codon 526, the author Mai Van Tuan mainly record that the His→Arg and His→Asp mutation patterns are slightly different from ours when the main results are His→ Leu (14.3%) [12]. Author Ngo Viet Quynh Anh also records that 32 strains (80%) of 40 strains of rifampicin resistant tuberculosis have mutations in rpoB codon 531. According to the results of other studies, rifampicin-resistant tuberculosis bacterial strains with a point mutation in the rpoB gene codon 531 is the most common (70% -73%), there are also some rifampicin-resistant strains that have mutations in this gene but at other codon positions such as codon 513 (27%) and codon 526 (7%) [2], [15].

Li J conducted whole-genome sequencing to predict drug resistance in multidrug-resistant tuberculosis in Tam Quyen, China from 2013 to 2019 with similar results to ours when it was recorded that rpoB was common in codon 526 with 3 strains, including 2 strains of H445L. In addition, this author also recorded results for rifampicin (RIF) ,all 12 strains carrying the RpoB mutation L430P also carried other RpoB mutations, the most common was at codon 435 (6 strains) [40]. Meanwhile, Gui-LianLi from Tianjin found that 58 out of 63 RFP-resistant isolates were found to carry mutations in the 81-bp RFP-resistance region (RRDR) of genrpoB and the common mutations were detected in codon 531 (44.4%),mutant TCG-TTG (Ser-Leu) was lower than in our study, but higher in codon group 526 (28.6%), specifically in 9 mutation types CAC-GAC (His-Asp), 5 mutations CAC-TAC (His-Tyr), 2 mutations CAC-CGC (His-Arg), 1 mutation CAC-CTC (His-Arg) and 1 mutation CAC-AAC (His-Asn) [37]

CONCLUSIONS

The rate of rifampicin resistance in Vinh Long Lung Hospital is quite high. It is found that there is an association between rifampicin resistance and history of TB treatment ($p<0.001$) and occupation ($p<0.019$). Mutation sites of the rpoB gene are found at codons 526 and 531 respectively.

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