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Research Article

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A SURVEY OF rpoB AND katG GENE MUTATIONS OF *Mycobacterium tubercolosis* ON PATIENTS WITH RIFAMPICIN RESISTANT PULMONARY TUBERCULOSIS IN VINH LONG LUNG HOSPITAL

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ABSTRACT

Introduction: Tuberculosis (TB) is currently a serious social disease due to its rapid spread within communities according to reports from the World Health Organization. Despite significant achievements in TB control in recent years, it remains one of the major public health issues globally. **Research Objective:** Determine the rate and types of rpoB and katG gene mutations in patients with pulmonary tuberculosis resistant to rifampicin and isoniazid in Vinh Long Lung Hospital. Research Subjects and Methods: A cross-sectional descriptive study is conducted on 412 eligible samples using Real-time PCR machines (Roto-Gene Q, Bio-Rad CFX96). **Results:** A study of 19 rifampicin-resistant samples reveals that 42.1% are multidrug-resistant (R+H), 31.5% are rifampicin-resistant only, and 5.2% are INH-resistant only. Mutations in the rpoB gene are detected in 73.7% of samples, primarily at codon 531 (S450L, 50%) and 526 (H445L, 21.4%). Mutations in the katG gene are found in 47.4% of INH-resistant samples with 88.9% at S315T position. The mutation rates for rpoB and katG genes are higher in new tuberculosis cases, but the differences are not statistically significant. **Conclusions:** The mutation rates of the rpoB and katG genes in rifampicin-resistant tuberculosis are 73.7% and 42.1%, respectively. rpoB mutations are concentrated at codons 526 and 531, while katG mutations predominantly occur at S315T. These findings provide a basis for understanding drug resistance mechanisms and support improvements in treatment strategies.

Keywords: Tuberculosis, Drug-resistant tuberculosis, rpoB mutation, katG mutation, Gene Xpert technique.

INTRODUCTION

Tuberculosis is still one of the most serious public health problems in the world with rapid spread and severe consequences. According to the World Health Organization (WHO), in 2019, there were approximately 10 million cases of tuberculosis, of which 8.2% were co-infected with HIV. Tuberculosis caused 1.2 million deaths including 208,000 deaths of people infected with HIV and it has been the leading cause of death from infectious diseases and has affected women most [15]. One of the biggest challenges today is drug-resistant tuberculosis, especially multidrug resistance to rifampicin and isoniazid, mainly due to mutations in the rpoB and katG genes[7]. Traditional diagnostic methods base on bacterial culture which takes 4-8 weeks to get results, slowing down the treatment process. However, with the advancement of biotechnology, tests such as Gene Xpert and gene sequencing have shortened the diagnostic time to 2 hours, providing high accuracy and supporting doctors in developing more effective treatment regimens [7]. This reality encourages us to conduct the topic "A survey of rpoB and katG gene mutations of Mycobacterium tuberculosis on patients with Rifampicin-resistant pulmonary tuberculosis in Vinh Long Lung Hospital".

SUBJECTS AND METHODS OF RESEARCH

Research subjects

Patients with pulmonary tuberculosis who had medical checks in Vinh Long Lung Hospital from June 2023 to July 2024.

Criteria for selecting samples

- Patients with Gene Xpert test results showing tuberculosis bacteria
- People aged 18 years or older
- Agree to participate in the study.

Exclusion criteria

- Patients who do not cooperate with paraclinical tests.
- Patients who cannot take sputum samples.
- Patients who do not agree to participate in the study.

Research methods

Research design: cross-sectional descriptive method.

Time and location of the study

From July 2023 to June 2024, in Vinh Long Lung Hospital.

Sample size

Apply the formula for calculating the estimated sample size of 1 proportion:

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

n: is the estimated sample for the study.

p: according to the research results of Hoang Ha (2021), the rate of rifampicin resistance in Thai Nguyen from 2016-2020 was 4%, choose p = 0.04% [7].

d: is the absolute error, choose d = 0.02.

α: choose the statistical significance level of 95%, with α = 0.05.

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$Z_{(1-\alpha/2)}$: is the value obtained by looking up the Z table, with $\alpha = 0.05$; $Z = 1.96$. Substituting into the formula, we get $n = 369$. In fact, our study collected 412 samples.

Sampling methods: choose convenient sampling according to the sampling criteria and exclusion criteria until the number of samples needed for the study is sufficient.

Research content

- General, clinical and paraclinical characteristics of the research subjects: Ages, genders, geography, living places, education levels, occupations, time of onset of illness, history of tuberculosis treatment, reason for hospitalization and blood count tests, liver and kidney function, chest X-ray characteristics of patients with drug-resistant tuberculosis.
- Determination of Rifampicin resistance rate detected by GeneXpert technique in pulmonary tuberculosis patients including GeneXpert results and DNA concentration of tuberculosis in sputum samples.
- Determination of rpoB and katG gene mutation rate on tuberculosis patients resistant to Rifampicin

Research equipment and data collection techniques

Research equipment: Sputum collection kit, TC Matrix automatic biochemical analyzer, Celltac Es Mek 7300k hematology analyzer, UD150L-40E fixed conventional X-ray machine, GenXpert machine system, RT-PCR CFX 96 Touch Detection System and accompanying equipment: centrifuge with rotor for 0.2ml PCR 8-strip tubes; 13,000rpm centrifuge with rotor for 1.5-2ml 12 or 2-strip tubes; Incubator with temperature control from $40^{0}C$ to $100^{0}C$; Vortex shaker; Vacuum pump; Micropitette ABI 3130XL automated gene-sequencing system with 16 capillaries, biological safety cabinet, heated shaker, minispin, conventional PCR machine, refrigerated centrifuge, capillary electrophoresis machine.

Data collection techniques: Data were collected through patient interviews by using a pre-written questionnaire; the collection tool was this questionnaire, which was revised and completed after a testing study on 10 patients.

Data analysis

- Using SPSS 20.0 software for statistical analysis. Collected data were coded and cleaned before being processed by SPSS 20.0 software.
- Analyzing sequencing results by Bio Edit software.
- Using descriptive statistical algorithms to describe variables.

Research ethics:

approved by the Ethics Council of Can Tho University of Medicine and Pharmacy.

RESEARCH RESULTS

Through research on 63 biopsy samples of patients positive for H. pylori bacteria, we obtained the following results:

General characteristics of the research subjects

Age Group

Table 1: Age distribution of research Subjects

Age group	Quantity (n)	Rate (%)
18 - 29	19	4,6
30 - 45	89	21,6
46 - 59	142	34,5
≥60	162	39,3
Total	412	100
The youngest age	18	
The oldest age	94	
The average age	**55,47±30,62**	

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

Gender

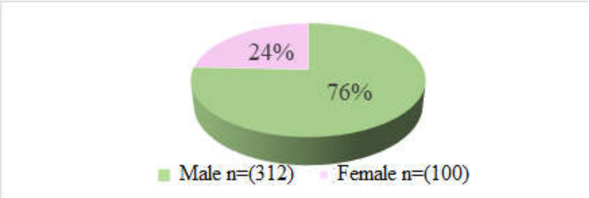

Figure 1: Distribution of research subjects by gender

Remarks: The majority of study subjects is male, accounting for 75.7%, 3 times higher than female (24.3%).

Rifampicin resistance rate detected by Gene Xpert technique in pulmonary tuberculosis patients.

Table 2: DNA concentration of tuberculosis in sputum samples

DNA concentration of tuberculosis	Quantity (n)	Rate (%)
Very low	72	17,5
Low	168	40,8
Average	118	28,6
High	54	13,1
Total	**412**	**100**

Remarks: The concentration of tuberculosis DNA in sputum samples has 4 levels, of which 40.8% of sputum samples have DNA concentration at low level, 28.6% of them have medium level, 17.5% of them have very low level and 13.1% of patients have high DNA concentration.

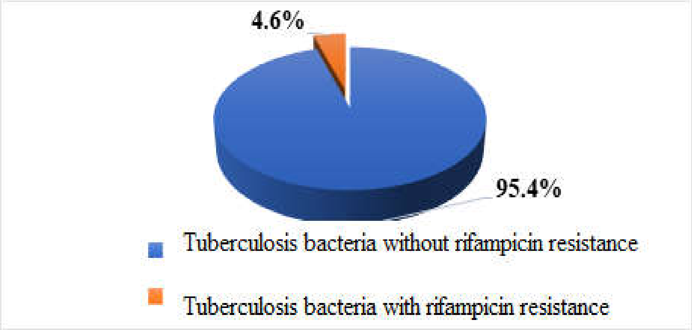

Figure 2. Rifampicin resistance rate is determined by Gene Xpert technique

Remarks: In 412 tuberculosis patients, Gene Xpert test detects 19 patients with rifampicin-resistant tuberculosis, accounting for 4.6%.

RpoB and katG gene mutation rate in patients with Rifampicin-resistant lung.

Table 3.20. Gene sequencing results

Gene sequencing results	Quantity	Rate
RIF-resistance	6	31,5
INH-resistance	1	5,2
Multidrug resistance (R+H)	8	42,1
Not found	4	21,1
Total	**19**	**100**

Remarks: Analysis of the gene sequencing results of 19 cases with Gene Xpert results of RIF resistance, of which there are 8 cases of RIF and INH resistance (42.1%), 6 cases of RIF resistance only (31.5%), 1 case of INH resistance only (5.2%). Especially, there are 4 cases (21.1%) without any RIF or INH resistance mutations when sequencing genes.

Table 3.21. Rate of rpoB gene mutations

rpoB gen mutations	Quantity (n)	Rate (%)
Found	14	73,7
Not found	5	26,3
Total	19	100

Remarks: Of the 19 RIF-resistant samples identified by Gene Xpert technique, the gene sequencing results find 14 cases with ropB mutations, accounting for 73.7%.

Table 3.22. RIF resistance mutation positions

Codon positions	Mutation positions	Acid amin	Quantity (N)	Rate (%)
511	L430P	Leu→Pro	0	0
512	S431G	Ser→Gly	0	0
513	Q432K	Gln→Lys	0	0
514	Q432L	Gln→Leu	0	0
516	D435G	Asp→Gly	0	0
	D435A	Asp→Ala	0	0
	D435V	Asp→Val	0	0
521	440 ins TCGGGGTTG	Ins TCGGGGTTG	0	0
526	H445N	His→Asn	2	14,3
	H445D	His→Asp	1	7,1
	H445Q	His→Gln	0	0
	H445S	His→Ser	0	0
	H445R	His→Arg	0	0
	H445L	His→Leu	3	21,4
	H445Y	His→Tyr	1	7,1
	H445F	His→Phe	0	0
531	S450L	Ser→Leu	7	50
533	L452P	Leu→Pro	0	0
561	I480V	Ile→Val	0	0
Total			**14**	**100**

Remarks: Sequence analysis of 14 RIF-resistant cases shows 2 mutated codon positions, 526 and 531, of which 50% are at S450L position of codon 531 (Ser→Leu), 21.4% are at H445L position of codon 526 (His→Leu). The mutation rate at position 531 and position 526 are both 50%. All 14 patient samples record RIF resistance mutations at only 1 codon position 526 or 531.

Table 3.23. Rate of katG mutations

katG mutation	Quantity (n)	Rate (%)
Found	9	47,4
Not found	10	52,6
Total	**19**	**100**

Remarks: The rate of katG mutations which are resistant to INH are found among 19 samples with 9 samples, accounting for 47.4%.

Table 3.24. INH resistance mutation positions

Mutation position	Quantity	Rate
S315T	8	88,9
Not found	1	11,1
Total	**9**	**100**

Remarks: In 9 cases with INH resistance, the majority of mutations is recorded at the katG gene (position S315T) accounting for 88.9%, and there is1 case (11.1%) without any katG mutation.

DISCUSSION

General characteristics of the research subjects

In our study, male patients account for 75.7%, 3 times higher than female patients (24.3%). This result is consistent with many domestic and international studies, showing that men are more likely to have tuberculosis due to heavy work, life pressure, smoking and drinking habits. Mai Van Tuan's study (2016) at Hue Central Hospital records the male/female rate of 66.4% and 33.6% [2]. Dang Vinh Hiep also shows that the rate of INH resistance in men is higher than in women (72.6% compared to 27.4%) [5]. Nguyen Thu Ha (2011-2012) records that men have 3-5 times higher rates of recurrent tuberculosis than women [1], [4]. However, a study by Le Hoan (2021) at Hanoi Medical University Hospital shows that the male/female rate is almost equal (54% and 46%) [6], suggesting that the difference may be due to the characteristics of the study population.

All patients in the study are over 18 years old, of which the ≥60-year-old group accounted for the highest proportion (39.3%), followed by 46-59-year-old (34.5%), 30-45-year-old (21.6%) and 18-29 year-old (4.6%). The average age is 55.47 ± 30.62. Compared with Mai Van Tuan [2], the age distribution in our study tends to increase with age. Meanwhile, Mohamed Muhumed Ali records a lower mean age (44.3 ± 14 years), with the 35-44-year-old group accounting for the highest rate (25.9%), and gradually decreasing in the over-64-year-old group (9.2%) [12].

Rifampicin resistance rate is detected by Gene Xpert technique in pulmonary tuberculosis patients

The overall RIF resistance rate of the tuberculosis bacteria samples we collect is 4.6%. This finding is consistent with the WHO Global Tuberculosis reports in 2020 and 2021, which records a low MDR/RR-TB rate in the Southeast Asia region of about 4% [13], [15].

The research results have a higher rate than many other domestic studies: Le Hoan's study on 1069 tuberculosis patients in Hanoi records a RIF resistance rate of 0.3%; Le Tien Cong studies the current situation of RIF-resistant tuberculosis in Thai Nguyen in the period of 2014 - 2022 and obtains the result of 1.91%; Meanwhile, author Nguyen Tran Thuy Anh records the result of RIF resistance at Pham Ngoc Thach Hospital, Ho Chi Minh City which is 3.13%; In foreign countries, according to author Orina Grace, the drug resistance test using Gene Xpert MTB/RIFT and DST show that 22 (3.23%) are resistant to RIF [9]. On the other hand, our results are much lower than that of author Mai Van Tuan who isolates the resistance rate of tuberculosis bacteria at 24.3% [2].

Regarding the overall DNA concentration in our study, it is mainly concentrated at low (40.8%) and medium (28.6%) levels. The results of this study are different from those of author Ha Thi Bich Ngoc when studying the application of GeneXpert/MTB/RIF technique in detecting tuberculosis bacteria in Hai Phong, recording DNA concentrations at relatively equal levels, respectively: very low (21.28%), low (30.85%), medium (29.79%) and high (18.09%) [3].

Rate of rpoB, katG mutations which were detected by gene sequencing method

Gene sequencing on 19 cases of rifampicin (RIF)-resistant tuberculosis by Gene Xpert shows the following distribution of drug resistance: 42.1% (8 cases) are resistant to both RIF and isoniazid (INH), 31.5% (6 cases) are resistant to RIF only, 5.2% (1 case) are resistant to INH only, and 21.1% (4 cases) are not detected with mutations resistant to RIF or INH. Mutation analysis of the rpoB gene reveals mutations in 73.7% (14/19 samples), with the most common positions at codon 531 (50%) and codon 526 (21.4%). There are no cases with mutations in two mentioned codons. Mutations in the katG gene are detected in 42.1% (8/19 samples), with 88.9% of these occurring at codon 315 - a position associated with INH resistance.

Compared with previous studies, the resistance rates and mutation positions differ, possibly relate to epidemiological factors, the prevalence of drug-resistant strains in the study area, or changes in antibiotic selection pressure over time. Notably, there are some cases where Gene Xpert shows RIF resistance but does not detect mutations in rpoB when sequencing. Probably, the reason is that the bacterial load in the sample is too low, affecting the accuracy of the test. This shows that caution is needed in interpreting Gene Xpert test results, especially in cases with low bacterial load or atypical results.

CONCLUSIONS

Rate of rifampicin (RIF) resistance detected by Gene Xpert in pulmonary tuberculosis patients in Vinh Long Lung Hospital is 4.6%, with a significantly higher risk in the group of recurrent tuberculosis patients and the group of civil servants. Analysis of gene mutations in RIF-resistant cases shows that 73.7% have rpoB mutations, most commonly at codons 526 and 531 (50% each), while 42.1% have katG mutations, mainly at position S315T (88.9%). This result confirms the association between history of tuberculosis treatment, occupation and drug resistance rate, and emphasizes the prevalence of gene mutations related to rifampicin and isoniazid resistance.

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