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Study On Clarithromycin And Levofloxacin Resistance Mutations In *Helicobacter Pylori* Bacteria Among Patients With Gastric Inflammation At Bac Lieu General Hospital

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ABSTRACT

Background: Helicobacter pylori infection in Vietnam accounts for 70-90% of the population. As the number one cause of inflammation, ulcers and stomach cancer, H. pylori bacteria can be effectively treated with antibiotics. However, drug resistance of H. pylori is a matter of great concern. **Objectives of the study:** To determine the rate of 23S rRNA and gyrA gene mutations resistant to clarithromycin and levofloxacin of H. pylori bacteria in gastritis patients and some related factors. **Subjects and methods:** A cross-sectional descriptive study on H. pylori-positive gastritis patients at Bac Lieu General Hospital. **Results:** The rate of mutations resistant to clarithromycin is (85.7%) and levofloxacin (60.3%). For clarithromycin, the mutation at position T2182C accounts for the highest rate (80.9%), A2143G (57.2%) and the lowest one is A2142G (1.6%). For levofloxacin, two common sites are amino acids 87 and 91, the rate of levofloxacin antibiotic resistance mutations for positions N87K, T87I, D91N, D91Y and D91G is (31.7%), (3,2%), (12.7%), (1.6%) and (14.3%) respectively. In particular, there is a newly discovered mutation capable of causing levofloxacin resistance is H (Histidine) at position 87H with the rate (1.6%). Up to 24 H. pylori samples have both levofloxacin and clarithromycin resistance mutations with the rate of 38.1%. **Conclusion:** The study has determined the rate of resistance mutations to the antibiotics clarithromycin and levofloxacin currently used to treat H. pylori, the mutation rate in the study is really remarkable and in need of attention in treatment.

Keywords: H. pylori, clarithromycin, levofloxacin, antibiotic resistance, gene sequencing

I. INTRODUCTION

According to the World Health Organization, *Helicobacter pylori* is the number one cause of inflammation, ulcers and stomach cancer. Since it was discovered and published in 1983 by Warren J.R. and Marshall B.J., *H. pylori* has attracted the attention of biomedical researchers. Currently, the prevalence of Helicobacter pylori infection accounts for more than half of the world's population, of which 25% is in developed countries and over 80% is in developing countries, and in Vietnam it accounts for 70-90% of the population. Vietnam also has the highest rate of stomach cancer among Southeast Asian countries, so successful eradication of *H.pylori* becomes essential to reduce the economic and disease burden caused by this bacterium. [7], [8], [12]. Recent studies have shown that Helicobacter pylori's resistance rate to clarithromycin and levofloxacin is increasing. Common causes of treatment failure can be poor acceptance of the drug, antibiotic resistance, and reinfection with *Helicobacter pylor*i. Among them, antibiotic resistance plays the most important role in increasing drug resistance in *H. pylori* bacteria. Poor drug acceptance may be due to the genetic nature of the patient, typically involving genes of the drug metabolism system in the body [11]. Today, with the development of science, especially in medicine, with the application of biotechnology and molecular biology in the diagnosis of *Helicobacter pylori* and treatment supervision, they have helped many patients. In order to contribute to the detection of *Helicobacter pylori* resistant to clarithromycin and levofloxacin antibiotics and effective treatment of gastric ulcers, we conducted a study on the topic "Study on clarithromycin and levofloxacin

resistance mutations in *Helicobacter pylori* bacteria among patients with gastritis patients at Bac Lieu General Hospital in 2022 – 2023" with the objective: "Determining the rate of mutations in the 23S rRNA and gyrA genes resistant to clarithromycin and levofloxacin of *H. pylori* among gastritis patients by Sanger gene sequencing and some related factors.

II. RESEARCH SUBJECTS AND METHODS

2.1. Research subjects

Patients who visited Bac Lieu General Hospital were diagnosed with gastritis, are indicated for endoscopy and biopsy samples in 2022-2023.

Sample selection criteria

- Patients diagnosed with gastritis have endoscopy, biopsy samples and chlorine test.
- Detection of positive *Helicobacter pylori* by PCR
- The patients consent to participate in the study

Exclusion criteria

- Pregnant and lactating patients
- History of gastric surgery.
- Zollinger-Eison syndrome
- Being treated with proton pump inhibitors, β-receptor blockers, EBMT, antibiotics for 1 month.

2.2. Research Methods

Reasearch design: cross-sectional description with analysis.

Time and place of the study

From January 2022 to December 2022, at Bac Lieu General Hospital

Sample size

According to a study by Tran Thien Trung et al. (2017), the rate of antibiotic-resistant mutations of CLA is 83.3%, LVX is 20% [19]. Corresponding to p = 0.833 and 0.2, we chose p = 0.2 to get the largest sample size. d: allowable error in the study d = 0.1.

Applying the formula for calculating sample size, the number of positive samples participating in the study is n = 62 samples.

The gyrA gene fragment of *Helicobacter pylori* updated by Genbank in 2021 has a length of 2487bp,

Primers for sequencing

On the gyrA gene segment, the gene region for levofloxacin resistance includes

Forward primer (5'-AGCTTATTCCATGAGCGTGA-3')

Reverse Primer (5'-TCAGGCCCTTTTGACAAATTC-3')

Analysis of the 23S rRNA gene to sequence the gene region that regulates clarithromycin resistance and primer pairs

Forward primer (5'-AGGTTAAGAGGATGCGTCAGTC-3')

Back primer (5'-CGCATGATATTCCCATTAGCAGT-3')

DNA after being extracted from gastric biopsy samples, the quinolone resistance gene region (QRDR) on the gyrA gene fragment and on the 23S rRNA gene fragment is amplified from the two corresponding primer pairs above, under the influence of DNA polymerase enzyme and under suitable conditions, cloned products are formed and sequenced using an automated DNA sequencing machine [6].

Sampling method: convenience sampling.

Research content

- General, clinical and endoscopic characteristics of research subjects: Age, sex, residence, education level, occupation, smoking, alcohol consumption and *H. pylori* treatment history, characteristics on endoscopic results, inflammatory and hurt patterns.
- Determination of the rate of clarithromycin and levofloxacin resistance mutations of Helicobacter pyori bacteria of different types, the rate of mutant genotypes.
- Factors related to resistance and mutation of clrithromycin and levofloxacin of *Helicobacter*

pylori bacteria: general factors of age group, sex, smoking, drinking alcohol, history of *Helicobacter pylori* treatment, place of residence, and factors on clinical features, lesion location, and type of lesion recorded through endoscopy.

Research facilities and data collection techniques

Research facilities: Endoscope, biopsy sample taking kit, biopsy sample collection kit, biopsy sample handling kit, biopsy sample extraction kit, refrigerator -2-14 °C, deep freezer -40 to -86°C sample transport container, testing machines: ABI 3130, Rotor-Gene – Quiagen, volumetric single channel Micropipette, centrifuge, vortex machine. Mixing shaker, Class II Biological Safety Cabinet and PCR preparation cabinet, 1.5/2.0 mL tube centrifuge, dry heater, nucleic acid/protein quantification spectrophotometer, Information collection appendices, stationery, computer.

Data collection techniques: use Appendix 1 to collect data and information of research subjects on: administrative information, clinical and subclinical characteristics. The research and data collection process includes research steps: data collection, collection of positive CLO biopsy samples, biopsy sample processing, DNA extraction, real-time PCR reaction, amplification of clarithromycin and levofloxacin antibiotic resistance, sending samples for gene sequencing, receiving and reading sequencing results, synthesizing results, entering data, processing data, analyzing data.

2.3. Data analysis

- Determine the presence of mutant forms by Bio Edit software.
- All data is encoded as variables
- The software uses SPSS 20.0 and is processed on the computer by statistical algorithms.

2.4. Research ethics: approved by the Ethics Committee of Can Tho University of Medicine and Pharmacy.

III. RESEARCH RESULTS

Through the study on 63 biopsies of patients who were positive for *H. pylori* bacteria, we obtained the following results:

3.1. General characteristics of the study subjects

3.1.1. Age group and gender

Table 1: Age and gender distribution of study subjects

Age group	Male		Female		Total	
	n	%	n	%	n	%
≤30	7	11,1	1	1,6	8	12,7
30-39	8	12,7	8	12,7	16	25,4
40-49	6	9,5	19	30,2	25	39,7
50-59	3	4,8	3	4,8	6	9,6
≥60	2	3,2	6	9,5	8	12,7
Total	26	41,3	37	58,7	63	100
Average age is 42,83 ± 13,81 years old						

Comment: The age group in the study subjects accounting for the highest rate is 40-49 (39.7%), the lowest is 50-59 (9.5%), the age group 30-39 accounts for 25.4%. The age ≤30 and ≥60 account for the same proportion of 12.7%. Subjects with gastritis indicated for endoscopy in women is 58.7%, men is 41.3%.

3.2. Results of clritromycin and levofloxacin resistance mutations of *H. pylori* by gene sequencing

Table 2: Rate of clarithromycin resistance mutations

Gene type	A2142G		Gene type	A2143G		Gene type	T2182C	
	n	%		n	%		n	%
A	62	98,4	A	27	42,8	T	12	19,1
G	1	1,6	G	36	57,2	C	51	80,9
Total	63	100	Total	63	100	Total	63	100

Comment: The T2182C mutation accounts for the highest percentage (80.9%), followed by A2143G (57.2%) and the last is A2142G mutation (1.6%). Up to (85.7%) samples are positive for the above mutations.

Table 3: Rate of levofloxacin resistance mutations

Positions	Amino acid	n	%
D86N	D / Aspartic acid	63	100
N/T87K/I/H	N / Asparagine	40	63,5
	K / Lysine	20	31,7
	I / Isoleucine	2	3,2
	H / Histidine	1	1,6
A88V/M	A / Alanine	63	100
D91/N/Y/G	D / Aspartic acid	44	71,4
	N / Asparagine	8	12,7
	Y / Tyrosine	1	1,6
	G / Glycine	10	15.8
A92M	A / Alanine	63	100

Comment: The two most common mutation sites in the sample population are positions 87 and 91. For position 87, the N87K mutation accounts for the highest percentage of 31.7%, followed by the T87I mutant form which accounts for only 3.2%. In particular, a new mutant form is discovered in the study, capable of creating resistance to levofloxacin of 87H, accounting for 1.6%. For mutation site 91, the mutation position D91N and D91G accounts for 12.7% and 15.8%, respectively, and the D91Y mutant form accounts for 1.6%.

3.3 Relationship between clarithromycin and levofloxacin resistance mutations with other characteristics

Table 4: Relationship between age group and clarithromycin resistance mutations

Age group	Clarithromycin resistance mutations		OR (95% CI)	p
	Yes (%)	No (%)		
<30	7(87.5)	1(12.5)	1.615(0.140-18.581)	0.700
30-39	13(81.3)	3(18.8)	0.609(0.048-7.758)	0.702
40-49	23(92)	2(8)	0.001(0.001)	0.999
50-59	6(100)	0	4.200(0.332-53.123)	0.268
≥60	5(62.5)	3(37.9)	Ref	-
Total	54(85.7)	9(14.3)		

Comment: The age group 40-49 has a higher rate of clarithromycin resistance mutations than the other groups. The difference is not statistically significant.

Table 5: Relationship between age groups and Levofloxacin resistance mutations

Age group	Levofloxacin resistance mutations		OR (95% CI)	p
	Yes (%)	No (%)		
<30	1(12.5)	7(87.5)	0.065(0.006-0.679)	0.022
30-39	11(68.8)	5(31.3)	0.080(0.008-0.762)	0.028
40-49	16(64.0)	9(36.0)	0.029(0.001-0.574)	0.020
50-59	5(83.3)	1(16.7)	0.048(0.003-0.665)	0.024
≥60	6(75.0)	2(25.0)	Ref	-
Total	39(61.9)	24(38.1)		

Comment: The age group 40-49 has a higher rate of levofloxacin resistance mutations than the other groups. The difference is statistically significant.

IV. DISCUSSION

4.1. General characteristics and endoscopic results of study subjects

Through our study (table 1), the average age of 63 gastritis patients in the study population is 42.83 ± 13.81 years old. We find that the average age in our study is approximately the one in these samples, higher than in some studies and lower than in others, although the difference was not significant. Our study is quite similar to that of Nguyen Thanh Nam in 2021, which is 40.24 ± 13.67 [2], Dao Thanh et al. in 2021 is 46.06 ± 13.95 [3]. The average age in our study is lower than some studies, the research of Park et al in 2020 is 60.5 ± 13.1 [9], Hanafiah et al in 2019 is 52.41 ± 16,44 [5], This difference may be due to the youngest age of patients included in the study, the youngest age in our study is 13 years old while the one in Park's study is 21 years old, and that of Hanafiah's study is 18. About age groups, our research shows that the age group 40-49 accounts for the highest rate of 39.7%, this rate is quite similar to the study of Nguyen Thanh Nam et al in 2021 which is 29.7 %.

Female patients in our study account for 58.7%, 41.3% higher than male patients. The results of our study are quite similar to that of some domestic authors in recent years, the rate of *Helicobacter pylori* infection in women is higher than that of men, the study of Nguyen Thanh Nam et al. in 2021 is 57.14%. 2], Nguyen Dang Khoa et al in 2021 is 56% [1].

4.2. Results of claritromycin and levofloxacin resistance mutations of *H. pylori* by gene sequencing

In our study by direct gene sequencing, it shows that the rate of clarithromycin resistance mutations is very high at 85.7%, some point mutations of the 23S rRNA gene at important positions such as: A2143G and A2142G account for 57.2%, 1.6% respectively and T2182C accounts for the highest proportion of 80.9%. The results of our study show that the rate is higher than that of Dao Thanh et al. in 2021 in Tien Giang with the rate of 77.5% [3], the clarithromycin resistance study sample, the rate of mutant forms is also lower than our study, A2143G and A2142G which are 68.1%,14.2% respectively and the other forms is 6.3%.

Research by Tran Nguyen Anh Huy et al (2021) has shown that the antibiotic resistance status of *H. pylori* bacteria in patients with gastritis - duodenitis in clarithromycin antibiotic resistant 94.8%, sensitive 5.2% and levofloxacin resistant 62.1%, sensitive 38.8% [6].

Tran Van Huy et al (2018) published a study to determine the prevalence of 23S rRNA gene mutations of *H. pylori* strain in Vietnam by PCR method. The A2143G mutation was detected in 36.1% of the samples, the A2142G mutation in 3.6%, while the A2142C mutation was not found in all cases [7].

Tang Le Chau Ngoc et al (2019) published a clarithromycin resistance mutation study of *H. pylori* at Children's Hospital 2 among 112 patients with gastritis caused by *H. pylori*. As a result, there was 95.3% of *H. pylori* clarithromycin resistance [9].

For levofloxacin resistance mutations, the study results show that the resistance rate is 61.9%. Our research is quite high compared to some other domestic and foreign studies. Tran Thien Trung et al in 2017 42.5% [4], Nguyen Dang Khoa et al in 2021 39.6% [1], Lok et al in 2020 in China 33.0% [6], Cui et al in 2021 in China 31.0% [11]. The mutation rates on the gyrA gene are 87 and 91, mainly N87K 31.7% and D91G 15.8%, D91N 12.7% the remaining forms in the study are found that T87I and D91Y account for 3.2%, 1.6%, respectively.

Tran Thi Nhu Le et al (2021) studied levofloxacin resistance mutations on gyrA, gyrB genes of *Helicobacter pylori* in patients with gastritis - duodenitis and recruited 65 patients with gastritis - duodenitis infected with *H. pylori*, of which 37/65 (56.9%) patients were resistant to levofloxacin [12].

In our study, we discover a new mutation that is capable of conferring resistance to levofloxacin at position 87H with the rate of 1.6%. The mutation rate in our study is quite similar to the study of Tran Thien Trung et al in 2017 with the mutation rate in the research population accounting for the proportion of mutant forms N87K, T87I, D91G, D91N, D91Y which are 22.6%, 3.8%, 7.5%, 7.5%, 3.8%, respectively [4], the above ratio shows that our study gives higher results than other studies. In previous studies, the difference could be due to geography, and it is found that resistance is increasing over time.

4.3. Relationship between clarithromycin and levofloxacin resistance mutations with other characteristics

In our study, the overall resistance rate of clarithromycin and levofloxacin in the age group 40-49 accounts for the highest rate, respectively 42.6%, 41.1%, followed by the age group 30-39, respectively 24.11%,28.2%. It is quite similar to Cui et al's study in 2021, the rate of resistance in the 40-49 age group was 36.9% clarithromycin-resistant and 30.3% levofloxacin-resistant. For the age over 50, the rate was 31.8% and 32.7% respectively [11], the study of Dao Thanh et al. in 2021, the mutation rate from 50 years old was 42.5%, under 50 years old was 57.4% [3]. Thus, it can be seen that the rate of mutations is higher in young people than in older people

CONCLUSION

The rate of mutations in the 23S rRNA and gyrA genes resistant to clarithromycin and levofloxacin of *H. pylori* is very high. In which, the rate of mutations resistant to clarithromycin is (85.7%) and levofloxacin (60.3%). For clarithromycin, the mutation at position T2182C accounts for the highest rate (80.9%), A2143G (57.2%) and the lowest one is A2142G (1.6%). For levofloxacin, two common positions are amino acids 87 and 91, the rate of levofloxacin antibiotic resistance mutations for positions N87K, T87I, D91N, D91Y and D91G is (31.7%), (3,2 %), (12.7%), (1.6%) and (14.3%). Specially, there is a newly discovered mutation capable of causing levofloxacin resistance. It is H (Histidine) at position 87H with the rate (1.6%). The increasing rate of drug-resistant mutations needs more attention in treatment.

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REFERENCE

1. Nguyen Van Khoa (2021), *Application of Etest techniques to determine drug resistance and gene sequencing techniques to detect levofloxacin resistance mutations of Helicobacter*

pylori bacteria in gastritis patients at Tien Giang Central General Hospital in 2020-2021, Master thesis in medical testing, Can Tho University of Medicine and Pharmacy
2. Nguyen Thanh Nam (2021), *Research on the detection of Helicobacter pylori bacteria in saliva by REAL-TIME PCR technique on patients with gastritis-duodenitis disease and inflammation at the Hospital of Can Tho University of Medicine and Pharmacy in 2020-2021*, Master thesis in medical testing, Can Tho University of Medicine and Pharmacy.
3. Dao Thanh (2021), *Application of REALTIME-PCR technique to detect clarithromycin resistance mutations of Helicobacter pylori in gastritis patients at Tien Giang Central General Hospital in 2020-2021*, Master thesis in medical testing, Can Tho University of Medicine and Pharmacy.
4. Tran Thien Trung, Tran Anh Minh, Nguyen Tuan Anh (2017), "Research on the rate of clarithromycin and levofloxacin resistance mutations of *Helicobacter pylori* by gene sequencing", *Vietnam Journal of Gastroenterology*, 9(49), p. 3074-3082.
5. Hanafiah A, Binmaeil H, Raja Ali R A, et al. (2019), "Molecular characterization and prevalence of antibiotic resistance in *Helicobacter pylori* isolates in Kuala Lumpur, Malaysia", *Infect Drug Resist*, (12) pp.3051-3061.
6. Tran Thi Nhu Le, Nguyen Van Lam, Tran Van De, Tran Ngoc Anh, Nguyen Vu Trung. (2022). Evaluation of the Antibiotic Resistance Rate of Helicobacter pylori in Peptic Ulcer Patients in Tien Giang Central General Hospital, Tien Giang Province, Vietnam, Open Access Maced J Med Sci. 2022 Aug 07; 10(B):1835-1839.
7. Lok H C, Zhu D, Wang J, et al. (2020), "Phenotype and molecular detection or clarithromycin and levofloxacin resistance in *Helicobacter pylori* clinical in beijing", (13) pp. 2145-2153.
8. Megraud F et al (2013), "*Helicobacter pylori* resistance to antibiotics in Europe and its relationship to antibiotic consumption", *Gut*, 62(1), pp. 34-42.
9. Murakami K et al (2009), "Sitafloxacin activity against *Helicobacter pylori* isolates, including those with gyrA mutations", *Antimicrob Agents Chemother*, 53(7), pp. 3097-3099.
10. Park J Y, Shin T S, Kim j H, et al. (2020), "The prevalence of multidrug resistance of *Helicobacter pylori* and Its impact on eradication in Korea from 2017 to 2019: A Single-center study", *Antibiotics (Basel)*, 9 (10), pp. 1-11.
11. Redondo J J et al (2018), "A novel RT-PCR for the detection of *Helicobacter pylori* and identification of clarithromycin resistance mediated by mutations in the 23S rRNA gene", *Diagn Microbiol Infect Dis*, 90(1), pp. 1-6.
12. Rongli Cui, Zhiqiang Song, Baojun Suoet al (2021) "Correlation Analysis Among Genotype Resistance, Phenotype Resistance and Eradication Effect of *Helicobacter pylori*" *Infect Drug Resist*, 14, pp. 1747-1756.
13. Venneman K et al (2018), "The epidemiology of *Helicobacter pylori* infection in Europe and the impact of lifestyle on its natural evolution toward stomach cancer after infection: A systematic review", *Helicobacter*, 23(3), pp. e12483.