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Primary antibiotic resistance of *helicobacter pylori* in vietnamese patients with peptic ulcer disease

TRAN THI KHANH TUONG[1]*, NGUYEN HONG THANH[1], AU NHAT HUY[2]
1. Pham Ngoc Thach University of Medicine 2. Faculty of Medicine, Tan Tao University

Abstract

Background: *Helicobacter pylori is a group 1 carcinogen. The genes cagA and vacA are the most studied, as they are crucial virulence factors linked to the pathogenicity of this bacterium. Antibiotic resistance is the main cause of failure in H.pylori eradication. Updating the antibiotic resistance situation of H.pylori in each region is important for recommending appropriate treatment regimens for the population and optimizing the effectiveness of H.pylori eradication treatment for each region and country. In addition, the polymorphism of the enzyme CYP2C19 alters the metabolism of proton pump inhibitors (PPIs), thereby affecting treatment outcomes in patients with peptic ulcer disease (PUD) and the eradication of H.pylori.* ***Objective:*** *To determine the rate of primary antibiotic resistance of H.pylori strains to Amoxicillin (AMX), Clarithromycin (CLA), Levofloxacin (LEV), Metronidazole (MET), and Tetracycline (TET) and investigate the prevalence of H.pylori virulence factors cagA, vacA and the phenotypes of CYP2C19 enzyme polymorphism.* ***Subject and method****: A cross-sectional descriptive study was performed on 216 patients at the American International Hospital from October 2019 to September 2022.Patients with peptic ulcer disease who underwent endoscopy and antibiotic culture by Epsilometer test (E-test) and the cagA, vacA genes, and CYP2C19 enzyme-encoding genes were identified using PCR techniques.* ***Result:*** *Among 216 patients, the mean age was 42.85 ± 11.76. The rate of primary resistance to CLA was the highest at 96.30%, while the rates of resistance to LEV, MET, and AMX were 58.8%, 8.80%, and 2.78%, respectively. In the study, there were no cases of primary antibiotic resistance to Tetracycline, with a resistance rate of 0%. There was no statistically significant difference in the rate of resistance to each antibiotic between the male and female groups (p>0.05). The rate of dual resistance to two antibiotics was very high, with 161/216 cases (74.54%). H.pylori strains with CagA (+) virulence factor accounted for 72.69%, while CagA (-) strains accounted for 27.31%. There was no significant gender difference in CagA distribution (p=0.488). All patients with peptic ulcer disease harbored H.pylori strains with VacA virulence, with the s1m1 type being the most common (50%). Patients with intermediate CYP2C19 metabolism phenotypes had the highest prevalence at 49.07%, followed by strong metabolizers (39.35%) and poor metabolizers (11.58%). There was no significant difference in CYP2C19 enzyme metabolism phenotypes between males and females (p=0.454).* ***Conclusion:*** *The very high rate of resistance to Clarithromycin indicates that the standard triple regimen is no longer effective and should not be used in clinical practice. The regimen with Levofloxacin should be combined with Bismuth to enhance treatment effectiveness. The*

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Corresponding author: *drkhanhtuong@pnt.edu.vn - Pham Ngoc Thach University of Medicine*

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rates of resistance to Amoxicillin, Tetracycline, and Metronidazole were low, so a Bismuth quadruple or a combination of these three drugs may be very effective in eradicating H.pylori. In untreated patients with H.pylori -induced peptic ulcer disease, CagA (+) genotype and VacA s1m1 genotype were predominant. These strains are associated with more severe tissue damage and higher activity levels compared to others. Patients with intermediate and strong CYP2C19 metabolizer phenotypes were relatively common. These patients may experience altered metabolism of proton pump inhibitors, which could impact treatment outcomes for peptic ulcer disease and H.pylori eradication.

Keywords: *Primary antibiotic resistance, Cag A, VacA,CYP2C19, H.pylori, peptic ulcer disease.*

I. Background

Helicobacter pylori (*H.pylori)* infection is one of the most common chronic bacterial infections in humans, affecting around 4.4 billion people worldwide [1]. It leads to various gastrointestinal issues, such as peptic ulcers and gastric cancer [1]. In 2009, the International Agency for Research on Cancer classified *H.pylori* as a Group 1 carcinogen [2]. To survive in the harsh acidic environment of the human stomach, *H.pylori* produces various virulence factor proteins, such as cagA, vacA, babA, sabA, and oipA. Among these, the cagA (cytotoxin-associated gene) and vacA (vacuolating toxin gene) have garnered the most attention due to their significant roles in the pathogenicity of this bacterium. Studies have shown that the inflammatory response in individuals infected with *H.pylori* strains expressing the CagA protein is higher than in those infected with CagA-negative strains. A more robust inflammatory response correlates with a higher risk of developing peptic ulcers and gastric cancer. CagA is regarded as the first oncoprotein identified in *H.pylori*. The prevalence of CagA-positive *H.pylori* strains is about 60-80% in Western countries and 90% in Asia. The vacA gene can induce cytotoxicity through vacuolation. Strains classified as s1/m1 are the most cytotoxic, followed by s1/m2 strains. Numerous studies have shown that individuals infected with vacA s1 or m1 strains have a higher risk of developing peptic ulcers and/or gastric cancer than those infected with s2 or m2 strains[3].

Eradicating *H.pylori* through treatment is essential in resolving many cases of peptic ulcers and preventing the development of gastric cancer. However, the increasing antibiotic resistance of *H.pylori* is a significant factor affecting the effectiveness of current treatment regimens and a leading cause of treatment failure. The antibiotic resistance situation of *H.pylori* tends to vary across different geographic regions and countries worldwide, including Vietnam. In Addition, the polymorphism of the CYP2C19 enzyme affects the metabolism of proton pump inhibitors (PPIs), which are a critical component of *H.pylori* eradication regimens. The frequency of CYP2C19 polymorphisms varies by population and ethnicity.

In Vietnam, research on the virulence factors CagA and VacA in *H.pylori*, as well as the polymorphism of the CYP2C19 enzyme in patients with peptic ulcers, remains limited. Therefore, we conducted this study with the objective of updating the current antibiotic resistance patterns and investigating the characteristics of the virulence factors CagA and VacA in *H.pylori* , and the distribution of CYP2C19 phenotypes in patients with *H.pylori* -induced peptic ulcers who have not yet undergone treatment.

2. Subject and method

The study will include all patients diagnosed with peptic ulcers in the gastrointestinal tract,

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infected with *H.pylori* at the American International Hospital from October 2019 to September 2022.

Inclusion criteria:

Patients aged ≥18 years with gastrointestinal symptoms and endoscopy-diagnosed peptic ulcers have not previously undergone *H.pylori* eradication therapy.

H.pylori strains isolated from patients will be subjected to antibiotic susceptibility testing against AMX, CLA, LEV, MET, and TET using the Epsilometer test (E-Test) method to determine minimum inhibitory concentrations (MICs). The *H.pylori* cultures will be grown on horse blood-enriched agar plates with antibiotics and incubated under microaerophilic conditions at 37 °C for 4 days.

H.pylori strains will be considered resistant when the MIC is ≥ 1 µg/ml for AMX, CLA, LEV, ≥ 8 µg/ml for MET, and ≥ 4µg/ml for TET.

Molecular Biology Method: DNA extraction was performed using the automatic Kingfisher-Flex system, followed by PCR techniques to detect specific *H.pylori* sequences in the urease gene and to determine the vacA and cagA genotypes of *H.pylori*.

CagA Virulence Factor: Positive/Negative.

VacA Virulence Factor: The vacA gene contains a signal region (s) and a middle region (m). The s region is divided into subtypes: s1 and s2, while the m region has subtypes m1 and m^2.

The polymorphism of the CYP2C19 enzyme alters the metabolism of proton pump inhibitors, potentially affecting the outcomes of *H.pylori* eradication therapy and peptic ulcer treatment. Genotyping was performed using real-time PCR to identify mutant alleles m1 on exon 5 and m^2 on exon 4 of chromosome 10, which encodes the CYP2C19 enzyme [4]:

Extensive Metabolizer (EM): Genotype wt/wt on exon 4 and exon 5.

Intermediate Metabolizer (IM): Genotype wt/m1 on exon 5 or wt/m^2 on exon 4.

Poor Metabolizer (PM): Genotype m1/m1 on exon 5 or m^2/m^2 on exon 4, or m1/wt on exon 5 and m2/wt on exon 4

Exclusion criteria:

Patients previously treated for *H.pylori* infection.

Patients with active gastrointestinal bleeding and Forrest IA, IB, IIA, IIB duodenal ulcers.

Patients with a history of gastric resection.

Research methods:

The study will employ a cross-sectional descriptive design. The sample size will be calculated using a specific formula.

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

The 2019 synthesis study by Vu Van Khiên and colleagues revealed the primary antibiotic resistance rates of *H.pylori* to AMX, CLA, MET, LEV, and TET were 15.0%, 34.1%, 69.4%, 27.9%, and 17.9%, respectively [5]. The required sample size (n) for each corresponding antibiotic resistance rate was calculated to be 49, 87, 82, 78, and 57, respectively. Therefore, we opted for the largest sample size, which requires at least n=87 patients.

The research protocol has been approved by the Biomedical Research Ethics Committee of Pham Ngoc Thach University of Medicine, under reference number 537/TĐHYKPNT-HĐĐĐ

3. Result

We have selected 216 patients. The general characteristics of the study subjects are presented in Table 1.

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Table 1. The general characteristics of the study population

Total number of patient	**N = 216**	
Age	42,85 ± 11,76	
Gender	Male	109 (50.5%)
	Female	107 (49.5%)
BMI	23,68 ±3,69	
History	Gastrointestinal ulcers	21.3%
	Gastroesophageal reflux	9.26%
	Diabetes	6.02%
	Hypertension	11.11%
	Family history of *H.pylori* infection	5.56%
	Family history of gastric cancer	2.78%
	Smoking	2.78%

Table 2. Endoscopic Findings

Lesion Type	**Frequency (n)**	**Percentage (%)**	**95% CI**
Gastritis	216	100	97.82 - 100
Gastric ulcer	33	15.28	10.87 - 20.83
Duodenal ulcer	23	10.65	7.01 - 15.74
GERD	78	36.11	29.78 - 42.94
Intestinal metaplasia (biopsy)	27	12.50	8.54 - 17.83

The rate of primary resistance to each type of antibiotics in H.pylori

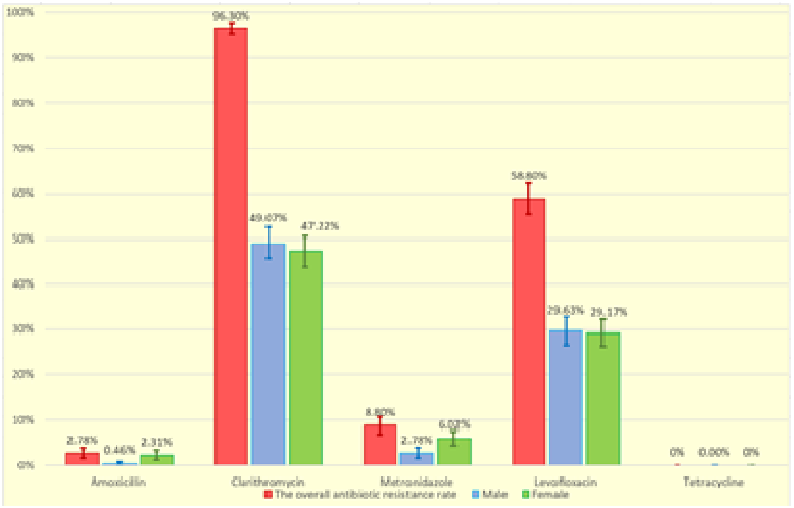

Figure 1. The rate of primary resistance to each type of antibiotics in *H.pylori* in the two groups of males and females

Comments: The rate of primary resistance to Clarithromycin is the highest at 96.30%, while there were no cases with primary resistance to Tetracycline (0%). The resistance rates to each type of antibiotic in both male and female groups showed no statistically significant difference, with p>0.05

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The rate of dual resistance of antibiotics

There were 161 cases of dual resistance of antibiotics, accounting for 74.54%

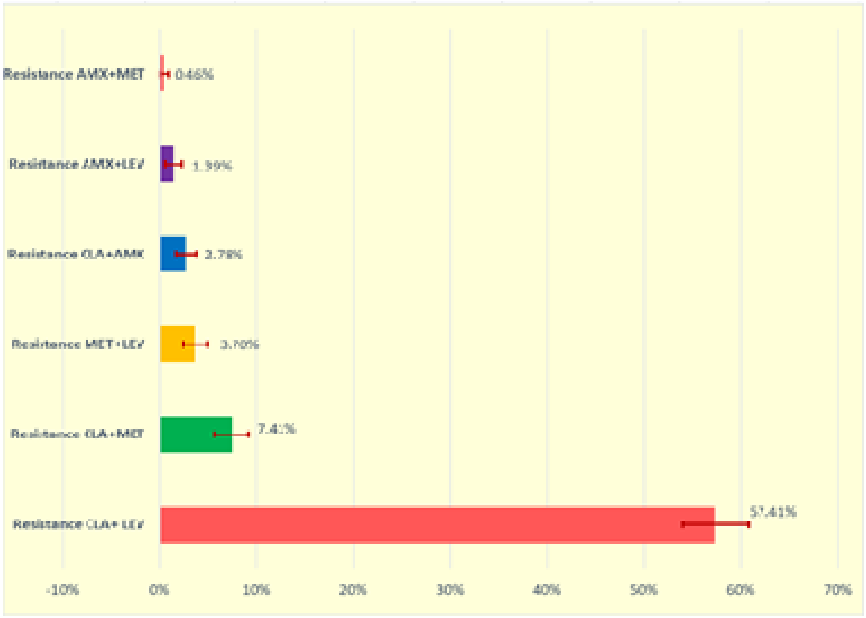

Figure 2. Resistance rate of *H.pylori* to 2 types of antibiotics

Comments: The rate of dual resistance of *H.pylori* to CLA+LEV is the highest, reaching 57.41%.

The resistance rate to 3 types of antibiotics

Table 3. The primary resistance rate to 3 types of antibiotics of *H.pylori*

Triple drugs resistance		**The number of drug-resistant strains**	**Percentage (%)**	**CI 95%**
CLA+MET+LEV	N=216	7	3.24	1.41-6.84
AMX+CLA+MET		1	0.46	0.02-2.95
AMX+CLA+LEV		3	1.39	0.36-4.33
Total		***11***	***5.09***	***2.7-9.17***

Comments: There are 11 cases of *H.pylori* strains that are resistant to 3 types of antibiotics, accounting for 5.09% of the total. Among them, there are 07 cases resistant to CLA+MET+LEV, which represents the highest rate at 3.24%.

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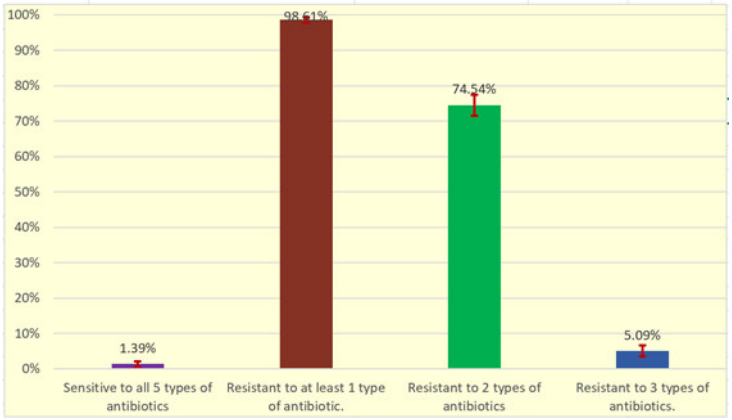

Figure 3. Chart of the distribution of antibiotic resistance rates

Comments: The primary resistance rate of *H.pylori* to at least 1 type of antibiotic accounts for 98.61% (213/216 cases). Resistance to 2 types of antibiotics represents 74.54%, while resistance to 3 types of antibiotics accounts for 5.09%. Only 1.39% (3 cases) remain sensitive to all 5 types of antibiotics in the eradication regimens. There are no cases showing primary resistance to 4 or more types of antibiotics, representing 0% of the cases

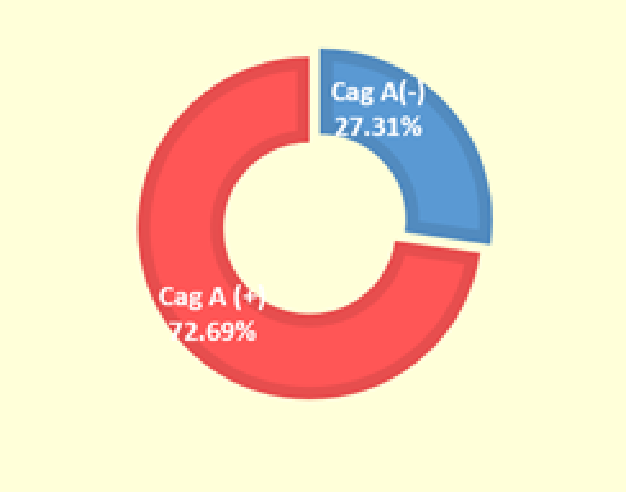

Figure 4. Distribution of *H.pylori* CagA Virulence Factor

CagA-positive *H.pylori* strains dominated, accounting for 72.69%, while CagA-negative strains made up 27.31%. There was no significant difference in CagA distribution by gender (p = 0.488).

Table 4. Distribution of CagA Virulence by Endoscopic Findings

Endoscopic Finding	CagA (+) n (%)	CagA (-) n (%)	p-value
Gastritis (n = 216)	157/216 (72.69%)	59/216 (27.31%)	-
Gastric ulcer (n = 33)	22/33 (66.67%)	11/33 (33.33%)	0.399
Duodenal ulcer (n = 23)	18/23 (78.26%)	5/23 (21.74%)	0.526
GERD (n = 78)	54/78 (69.23%)	24/78 (30.77%)	0.3
Intestinal metaplasia (n = 27)	25/27 (80.76%)	2/27 (19.24%)	0.011

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CagA-positive strains were more prevalent in cases of gastric ulcers, duodenal ulcers, and GERD. There was a statistically significant difference in the CagA (+) rate in patients with intestinal metaplasia (p=0.011).

Table 5. Distribution of CagA Virulence by Antibiotic Resistance

Antibiotic Resistance	CagA (+) n (%)	CagA (-) n (%)	p-value
Amoxicillin (n=6)	4/6 (66.67%)	2/6 (33.33%)	0.737
Clarithromycin (n=208)	151/208 (72.60%)	57/208 (27.40%)	0.881
Metronidazole (n=19)	14/19 (73.68%)	5/19 (26.32%)	0.918
Levofloxacin (n=127)	97/127 (76.38%)	30/127 (23.68%)	0.146

There was no statistically significant difference in the distribution of CagA-positive and CagA-negative strains across different antibiotic resistance profiles (Chi-squared and Fisher's tests).

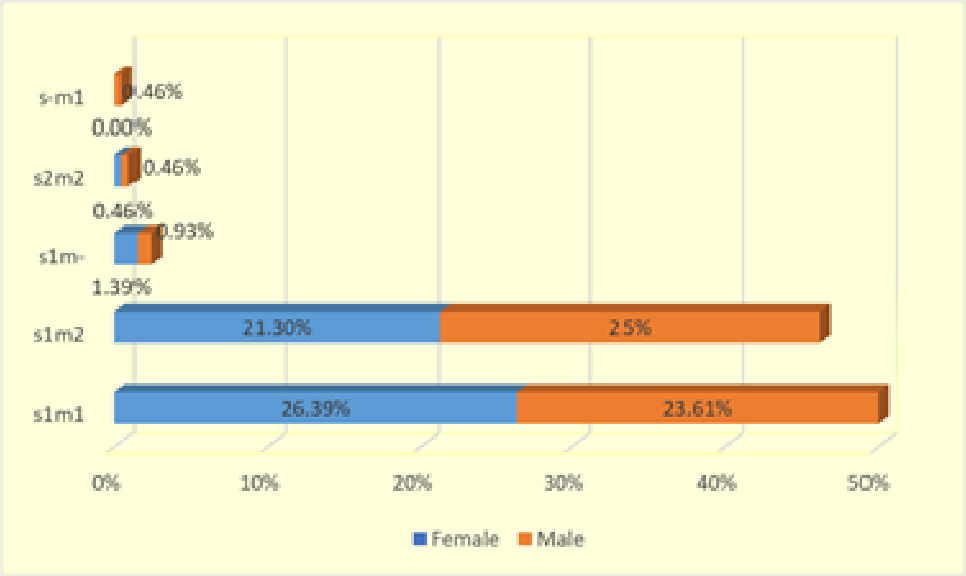

Figure 5. Distribution of *H.pylori* VacA Virulence by Gender

VacA-positive *H.pylori* strains were present in 100% of patients, with the s1m1 genotype being most common at 50%. There was no significant gender difference in VacA distribution (p=0.78, Fisher's test).

Table 6. Distribution of VacA Virulence by CagA Status

VacA Genotype	CagA (+) n (%)	CagA (-) n (%)	p-value
s1m1	92 (42.59%)	16 (7.41%)	0.003
s1m2	61 (28.24%)	39 (18.06%)	
s2m2	0 (0%)	2 (0.93%)	
s-m1	1 (0.46%)	0 (0%)	
s1m-	3 (1.39%)	2 (0.93%)	
Total	157/216	59/216	

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There was a significant difference in the distribution of VacA virulence by CagA status. Among CagA-positive strains, the VacA s1m1 genotype predominated (42.59%), while in CagA-negative strains, the VacA s1m2 genotype was most prevalent (18.06%) (Chi-squared and Fisher's tests).

CYP2C19 Allele Frequencies on Exon 4 and Exon 5

Table 7. CYP2C19 Allele Mutation Frequencies on Exon 4 and Exon 5

Allele Type	Frequency	Percentage (%)	95% CI
Wild-type (wt) allele	300	69.44	64.83 - 73.71
Mutation on exon 5 (m1)	114	26.39	22.35 - 30.36
Mutation on exon 4 (m2)	18	4.17	2.56 - 6.63
Total (216 patients, 432 alleles)	432	100	

Table 8. CYP2C19 Phenotype Distribution

Metabolizer Phenotype	Frequency (n)	Percentage (%)	95% CI
Extensive (EM)	85	39.35	32.86-46.23
Intermediate (IM)	106	49.07	42.25-55.93
Poor (PM)	25	11.58	7.77-16.7
Total	216	100	

IM phenotype was the most prevalent (49.07%), followed by EM (39.35%) and PM (11.58%). No significant gender difference in CYP2C19 metabolism phenotypes was observed (p=0.454).

4. Discussion

The average age in our study was 42.85 ± 11.76, with the oldest participant being 74 years old and the youngest being 18 years old. There were 107 females, accounting for 49.5% of the participants, and 109 males, accounting for 50.5%. The male-to-female ratio was 1.02/1. The mean body mass index (BMI) in the study was 23.68 ± 3.69. The rate of overweight and obesity was 55.1%. Several meta-analyses of various disease studies have found a positive correlation between *H.pylori* infection and the development of obesity. Therefore, *H.pylori* -positive patients were more likely to be obese, and obese individuals had a higher risk of *H.pylori* infection [6].

The research results showed that the prevalence of primary resistance to CLA was the highest, reaching 96.30%, while the resistance rates to LEV, MET, and AMX were 58.8%, 8.80%, and 2.78%, respectively. No cases of resistance to TET were found in the study, with a resistance rate of 0%. When comparing these findings with other studies on *H.pylori* antibiotic resistance conducted in different regions and countries over the years, our research revealed some differences, which are presented in the table.

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Table 9: Prevalence of primary antibiotic resistance in various domestic and international studies.

Domesetic studies						
Author/n	**Year/Region**	**Prevalence of primary antibiotic resistance%**				
		AMX	**CLA**	**MET**	**LEV**	**TET**
Tran Thanh Binh (n=103) [7]	2013 South/North	0	33	69.9	18.4	5.8
Phan Trung Nam (n=92) [8]	2015/Central region	0	30.2	67.4	39.5	-
Quek Camelia (n=57) [9]	2016/ South	5.3	87.7	47.4	36.8	24.6
Đang Ngoc Quy Hue [10] (n=119)	2018/South	-	66.1	-	37.8	-
Vu Van Khien [5] (10 studies)	2019/ All	15	34.1	61.5	45.7	23.5
Our study /(n=216)	2022/ South	2.78	96.30	8.80	58.8	0
International studies						
Author/n	**Year/ Nation**	**Prevalence of primary antibiotic resistance%**				
		AMX	**CLA**	**MET**	**LEV**	**TET**
Shiota S [11] (n=135)	2015/US	0	16.4	20.3	31.3	0.8
Yu-Ting Kuo [12](176 studies)	2016 Asia-Pacific region	3	17	44	18	4
Bachir M (n=151) [13]	Algeria	0	22.8	61.1	0	-
Dan Wang (N=100) [14]	2019/China	9	31	78	56	15
Palmitessa V (N=92)[15]	2020/ Italy	1.6	37.7	16.4	26.2	0

The prevalence of primary CLA resistance in our study is quite high compared to national studies conducted between 2013-2019. However, we believe this is consistent with the current situation in Vietnam, where CLA, a widely used Macrolide antibiotic, is commonly prescribed by doctors and easily accessible to the public without a prescription for treating common infections such as upper respiratory tract infections, pharyngitis, etc. The high resistance rate of 96.3% indicates that the current standard triple therapy is no longer highly effective in eradicating *H.pylori.*

The prevalence of primary LEV resistance in our study is 58.8%, which is higher than other domestic studies. However, when considering the trend over time, it shows that the prevalence of LEV resistance is gradually increasing. In the years 2013, this rate was only about 18%, but it steadily increased over 5 years to approximately 39% in 2018, as observed in Dang Ngoc Quy Hue's study. Continuing the trend, in our study conducted in 2022, the LEV resistance rate reached nearly 60%, surpassing the rates reported in other studies worldwide and in the region. According to Salvodi's meta-analysis study in

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2018, the prevalence of primary LEV resistance in the Southeast Asia region was 30%[16].

The prevalence of primary MET resistance in our study is only 8.8%, significantly lower than previous domestic studies. About 10 years ago, Tran Thanh Binh's study showed a MET resistance rate of nearly 70%. In our study, the prevalence was even lower at around 8.8%. In a long-term cohort study conducted in Spain by Ana Morilla over 13 years, the results indicated a decreasing trend in MET resistance, from 45% in 2004 to 30% in 2015. This suggests that unlike the increasing trend observed with other antibiotics, MET resistance tends to decline over time.

MET is a type of antibiotic belonging to the Nitroimidazole group and is used to treat anaerobic bacterial infections. It is usually prescribed and used by specialized doctors. Strict adherence to the prescribed antibiotic regimen and proper treatment duration can contribute to limiting the development of *H.pylori* bacterial mutations and antibiotic resistance.

The prevalence of primary AMX resistance in our study is approximately 2.78%, which closely aligns with the findings of other studies. Yu-Ting Kuo reported a resistance rate of 3%, Quek Camelia found a rate of 5.3%, while Tran Thanh Binh and Phan Trung Nam's study did not identify any *H.pylori* strains resistant to AMX. Other studies conducted worldwide and in the region have also shown very low rates of AMX resistance. According to Salvodi's meta-analysis study in 2018, the prevalence of primary AMX resistance in the Southeast Asia region was 2% [16], which is consistent with our findings. These results collectively suggest that AMX remains an effective antibiotic for treating *H.pylori* infections, and the prevalence of AMX resistance is relatively low in both local and global settings

In our study, no cases of primary TET resistance were detected. In Vietnam, Tran Thanh Binh's study in 2013 reported a similar rate of approximately 5.8%. However, in studies conducted by Quek Camelia, the prevalence of primary TET resistance showed an increasing trend, reaching nearly 25%.

The results of our study are consistent with the findings of Bachir [13] and the meta-analysis conducted by Salvodi in 2018 [16], both of which reported a 0% prevalence of primary TET resistance in the Southeast Asia region and Italy [15], respectively. TET remains a highly sensitive antibiotic against *H.pylori* in Vietnam, possibly due to its limited use and rarity in community treatments, leading to fewer occurrences of bacterial mutations. Additionally, our study found no statistically significant differences in resistance rates between male and female participants, which is in line with Tran Thanh Binh's study [7].

The results reveal a very high dual resistance rate to two types of antibiotics, accounting for 161 out of 216 cases (74.54%). This rate is higher than the findings from Tran Thanh Binh's study in 2013 [7], where the dual resistance rate to two antibiotics was 57.28%, and Dan Wang's study [14], which reported a rate of 23%. This is a concerning figure that highlights the alarming state of multi-drug resistance in *H.pylori.* Among the dual resistance cases, the highest rate was observed for CLA + LEV accounting for 124 cases (57.41%). This rate significantly increased and showed a statistically significant difference ($p<0.001$) compared to Tran Thanh Binh's study (8.7%). Regarding the CLA+ MET dual resistance, the rate decreased from 24.3% in Tran Thanh Binh's study in 2013 to 7.41% in our study in 2022, which is a statistically significant reduction ($p=0.006$). This improvement could be attributed to the decrease in the single resistance to MET, which dropped to 8.80% in our study, compared to 69.9% in Tran Thanh Binh's study [7]. Additionally, our study did not identify any cases of dual resistance to MET+ TET which is

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an encouraging signal and aligns with the treatment recommendations for *H.pylori*, particularly the first-line quadruple therapy that includes Bismuth, MET, and TET as the main antibiotics [17].

In our study, we observed 11 cases of triple resistance to three types of antibiotics, accounting for 5.09% of the cases. However, no cases were found to have resistance to four or more types of antibiotics. This triple resistance rate is lower than that reported in Tran Thanh Binh's study, where the triple resistance rate was 14.56%, and resistance to four types of antibiotics was found in 2 out of 103 cases (1.9%) [7]. Our study also revealed that only about 1.39% of H.*pylori* strains were susceptible to all five types of antibiotics tested, indicating that up to 98.61% of *H.pylori* strains were resistant to at least one type of antibiotic. This finding is similar to the study by Dan Wang, which reported a 1.9% rate of strains being susceptible to all five antibiotics [14]. The low rate of H.pylori strains remaining sensitive to all five antibiotics highlights the importance of using combination therapy in the eradication of *H.pylori* infections. Relying on a single antibiotic may not be effective due to the high prevalence of antibiotic resistance. In the future, it is hoped that clinical researchers will discover and develop new antibiotics to add to the treatment protocols for *H.pylori*, providing more effective options for eradicating the bacteria.

Among 216 *H.pylori* strains successfully cultured, CagA (+) strains were predominant (72.69%), while CagA (-) strains accounted for only 27.31%. Similar findings were observed in other studies with CagA (+) prevalence ranging from 71% to 91% in different regions.

Results from 216 successfully cultured *H.pylori* samples showed that strains with CagA (+) virulence predominated at 72.69%, while strains with CagA (-) virulence accounted for only 27.31%. This result is similar to the study by Thái Thị Hồng Nhung, which found a CagA(+) rate of 83.8% [18], Trần Thiện Trung's study with 91.3% [19], Iran with 76%, and Iraq with 71% [20]. It is higher than Rania's study at 53% [21] but lower than the study in Japan where CagA accounted for about 90% and was correlated with a high rate of cancer in that country [22]. The study results were higher than those in Algeria, where CagA (+) was 58% in patients [23], 53% in Egypt [21], and 24.2% in Pakistan[24]. Previous studies have shown that the CagA (+) rate in *H.pylori*-infected patients is usually higher in Asians compared to Europeans and Africans. This is also a disadvantage as these are highly virulent *H.pylori* strains, causing a higher inflammatory response in CagA (+) strains compared to CagA (-) strains. The higher the inflammatory response, the greater the risk of peptic ulcer disease (PUD) and gastric cancer. The rate of CagA (+) in patients with intestinal metaplasia was significantly different from CagA (-) with p=0.011. Intestinal metaplasia is a precancerous lesion with a risk of developing into gastric cancer. Previous studies have shown that the CagA (+) gene is found more frequently in gastric cancer patients than in controls [19]. CagA is considered the first oncoprotein of H.pylori [25].

H.pylori strains with VacA virulence accounted for 100% in the group of PUD patients, with the s1m1 type being the most prevalent at 50%. The s1m2 and s2m2 types accounted for 46.3% and 0.93%, respectively. In our study, the s1m1 and s1m2 genotypes accounted for up to 96.3%, higher than the other genotypes, similar to Lê Quý Hưng's study on gastric cancer patients where the s1m1 and s1m2 genotypes accounted for 84.4% [26]. Similarly, in Algeria, the s1m1 genotype was the most common at 59.88% [23]. There was a significant difference in the distribution of VacA virulence between the CagA (+) and CagA (-) groups. In the CagA (+) group, the s1m1 type predominated at 42.59%, while in

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the CagA (-) group, the s1m2 type was more common at 18.06%. This result is similar to Rania's study, where the *H.pylori* strain with CagA+/VacA s1m1 virulence was the most predominant (26/60, 43.3%) [21]. This is consistent with Memon Ameer's findings, where the s1m1 genotype is the most common in the Asian population [24]. This genotype is also prevalent in Brazil, Mexico, and Latin America [27]. The s1m1 strains are the most cytotoxic, followed by the s1m2 strains. Many studies in Western countries, including Latin America, the Middle East, and Africa, have shown that individuals infected with VacA s1 or m1 strains have a higher risk of PUD and/or gastric cancer compared to those infected with s2 or m2 strains [28].

The CYP2C19 enzyme gene is located on chromosome 10. The wild-type allele, designated as wt or CYP2C19*1, has full enzyme activity. The m1 mutation on exon 5 is known as CYP2C19*2, and the m2 mutation on exon 4 is known as CYP2C19*3, both of which result in loss of enzyme activity. Study results in Table 5 showed that non-mutant alleles had the highest frequency at 69.44%, mutations resulting in loss of CYP2C19 enzyme activity mainly occurred on exon 5 at 26.39%, while mutations on exon 4 accounted for 4.17%. Similarly, the study by Tran Phuong showed non-mutant alleles at 65.34%, mutations causing deletions on exon 5 at 28.69%, and mutations on exon 4 at 5.97% [29]. In Wichittra's study, the CYP2C19*1, CYP2C19*2, and CYP2C19*3 rates in the Thailand population were 68%, 29%, and 3%, respectively, while in the Myanmar population they were 66%, 30%, and 4%, respectively [30]. This comparison shows that the allele frequency distribution in the Vietnamese population is quite similar to that of other Asian populations. In Asia, the CYP2C19*2 frequency ranges from 25-36% and CYP2C19*3 from 2.5-10%, significantly higher than in Europe where the CYP2C19*2 frequency is 15% and CYP2C19*3 is 0.02% [31].

The CYP2C19 enzyme is known to metabolize many drugs, especially PPIs. PPIs play a crucial role in controlling stomach pH and eradicating *H.pylori*. CYP2C192 and CYP2C193 mutations reduce enzyme activity, leading to decreased drug metabolism and PPI elimination, potentially benefiting and enhancing the treatment effectiveness of *H.pylori*. According to Goldstein, if there are no mutations (wt/wt), the phenotype will be an extensive metabolizer (EM), meaning the enzyme activity is not reduced. If only one mutant allele is present (wt/m1 or wt/m2), the enzyme phenotype will be an intermediate metabolizer (IM), meaning the enzyme activity is partially reduced. If both alleles are mutant (m1/m1, m2/m2, or m1/m2), the enzyme phenotype will be a poor metabolizer (PM), indicating significantly reduced enzyme activity[4].

Results in Table 8 showed that PUD patients had the highest proportion of IM phenotype at 49.07%, followed by EM at 39.35%, and PM at the lowest at 11.58%. There was no significant difference in CYP2C19 enzyme phenotype distribution between males and females (p=0.454). Similarly, Phan Trung Nam's study found that the IM rate was the highest at 47.5%, EM at 41.5%, and PM at 11% [32], and Trần Ngọc Lưu Phương's study showed that the IM rate was the highest at 49%, followed by EM and PM at 43.43% and 7.75%, respectively [29]. Similarly, Li He's study in China found IM, EM, and PM rates of 45.62%, 40.96%, and 13.42%, respectively [33]. This differed from Rattanaporn.S's study in Thailand, where the IM, EM, and PM rates were 42.98%, 50.82%, and 6.64%, respectively [34]. The IM, EM, and PM rates in Caucasians were 69%, 27%, and 3%, respectively, while in Africans, the rates were 62%, 32%, and 4%, respectively [35]. Comparing

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studies, we found that Vietnamese and Asian populations generally have a predominance of the IM phenotype, followed by EM, and PM accounting for about 10%. In contrast, European and African populations have a predominance of the EM phenotype (usually over 60% of the population), with the IM phenotype accounting for about 30% and the PM phenotype about 5%. Vietnamese and Asians have a higher advantage in drug metabolism mutations compared to Europeans and Africans. Differences in the CYP2C19 enzyme's metabolism level for PPIs affect drug concentration in the blood, thereby impacting treatment effectiveness.

4. Conclusion

Sensitivity testing to antibiotics is highly important in determining the appropriate eradication regimen for each patient with *H.pylori* infection. The high resistance rate to CLA indicates that the standard triple therapy is no longer effective and should not be used in clinical practice.

Regimens containing LEV should be combined with Bismuth to enhance treatment efficacy. On the other hand, the low resistance rates to AMX, TET, and MET suggest that the 4-drug regimen with Bismuth or the triple therapy combining these three antibiotics together is highly effective in eradicating *H.pylori*. Selecting the appropriate treatment regimen based on sensitivity testing results will improve treatment efficacy and prevent antibiotic resistance.

H.pylori strains with CagA (+) virulence predominated at 72.69%, while those with CagA (-) virulence accounted for only 27.31%. *H.pylori* strains with VacA virulence were present in 100% of the PUD patient group, with the s1m1 type being the most prevalent at 50%. There was a significant difference in the distribution of VacA virulence between the CagA (+) and CagA (-) groups. *H.pylori* strains with the CagA (+) genotype and VacA s1m1 genotype are associated with more severe tissue damage and higher activity levels compared to other strains. Among PUD patients, those with intermediate CYP2C19 metabolizer phenotypes accounted for the highest percentage at 49.07%. Patients with strong metabolizer phenotypes may experience altered metabolism of PPIs, which could affect the treatment outcomes for PUD and the eradication of *H.pylori*.

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