Characterization of antibiotic resistance and genotyping of *Helicobacter pylori* isolates in patients with gastroduodenal disease

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Abstract

Background: In this study, we assessed the status of antibiotic resistance of *H. pylori* isolates to antimicrobial agents at Hospital of Hue University of Medicine and Pharmacy in Central Vietnam and identified the underlying molecular mechanisms related to tetracycline and amoxicillin resistance. In addition, the *cagA*, *cagE*, *cagT*, *vacA*, *iceA* genotypes of *H. pylori* strains were investigated to predict clinical outcomes. **Materials and methods:** *H. pylori* was successfully cultured in 52 patients with different gastrointestinal disorders at the Hospital of Hue University of Medicine and Pharmacy in Central Vietnam. The minimum inhibitory concentrations (MICs) of five antimicrobials; clarithromycin (CLR), metronidazole (MTZ), levofloxacin (LE), amoxicillin (AMX) and tetracycline (TE) were determined by the E-test method. Genetic determinants of AMX and TE resistance were identified with the polymerase chain reaction (PCR), followed by sequencing and gene analysis. Allelic variants of *cagA*, *cagE*, *cagT*, *vacA*, *iceA* were identified by the PCR. **Results:** The resistance to CLR, MTZ, LE, AMX, and TE were 90.4%, 86.5%, 65.4%, 40.4% and 0%, respectively. Multidrug resistance was observed in 88.5% of the isolates investigated. Several known AMX resistance mutations were identified in PBP1A (A369T, V374L, S543R, T556S, N562Y), whereas a known mutation in 16S rRNA (A926G) was detected in strains with higher MIC level (TE MICs of 0.25 and 0.5 mg/L). The *cagA, cagE* and *cagT* genotypes were found together in 46 isolates (88.5%), *vacA*s- region genotype in 51 (98.1%, predominantly *vacA*s1), *vacA*m-region genotype in all strains studied (*vacA*m1 - 51.9%, *vacA*m2 - 40.4%, *vacA*m1 and *vacA*m2 - 7.7%), *iceA1* in 22 (42.3%) and *iceA2* in 20 (38.5%) of strains. The allelic variant *vacA*s1m1 was prominent (57.4%), and *vacA*s1m2 (42.6%). **Conclusion:** Overall, resistance rates to CLR, MTZ, LE and AMX were high in *H. pylori* from Central Vietnam, except for TE, which serves as a foundation for developing local guidelines for more effective therapeutic strategies. Neither the single genes nor the combination of genes was significantly helpful in predicting the clinical outcome of *H. pylori* infection in patients in our study.

Key words: *H. pylori, antibiotic resistance, genotype.*

1. BACKGROUND

Helicobacter pylori (*H. pylori*) infection almost always results in chronic gastritis, with only a small but significant proportion of infected individuals developing severe inflammation leading to peptic ulcer disease (PUD), even gastric carcinoma and gastric mucosa-associated lymphoid tissue lymphoma. The successful eradication of *H. pylori* is important for preventing and treating gastroduodenal disease. The antimicrobials most widely used for *H. pylori* therapy include clarithromycin (CLR), metronidazole (MTZ), levofloxacin (LE), amoxicillin (AMX) and tetracycline (TE). The resistance rate has continued to increase worldwide and varies significantly according to the geographic region [1]. Therefore, Maastricht V Consensus Report recommends that local surveillance of *H. pylori* antibiotic resistance is mandatory to select appropriate eradication regimens according to local resistance patterns [2]. Phan et al. conducted an evaluation of antibiotic resistance to the four commonly used antibiotics against *H. pylori* at a tertiary care hospital in Central Vietnam from 2012 - 2014 and showed that resistance rates of *H. pylori* to CLR, LE, MTZ and AMX were 34.1%, 27.9%, 72.0% and 1.1%, respectively [3]. Previous studies have shown that certain point mutations in 16S rRNA confer resistance to TE; meanwhile, some amino acid substitutions in penicillin-binding protein 1A (PBP1A) confer AMX in *H. pylori* [4], [5]. Although *H. pylori* infection is widespread worldwide, most infected individuals remain asymptomatic. The clinical outcome of *H. pylori* infection is most likely

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determined by complex interactions between *H. pylori*, the host, and environmental factors. In particular, several virulence genes, such as the cytotoxin-associated gene (*cag)A, cagE, cagT* genes, the vacuolating cytotoxin (*vac)A* gene, and the induced by contact with epithelium (*ice*)*A* gene have been reported to play an important role in the pathogenesis of *H. pylori* infection. The objectives of our study were aiming:

1. To evaluate the status of H. pylori resistance to antibiotics in the patients with peptic disease coming for examination and treatment at Hospital of Hue University of Medicine and Pharmacy in the Central region of Vietnam.

2. To characterize phenotypically and genotypically tetracycline, amoxicillin-resistant H. pylori isolates.

3. To detect certain cagA, cagE, cagT, vacA, iceA genotypes of H. pylori isolated from patients with gastroduodenal disease such as chronic gastritis and peptic ulcer disease.

2. MATERIALS AND METHODS

2.1. Study design:

This was a cross-sectional study.

2.2. Ethics statement: This study was approved by the Instutional Ethics Committee (IEC) of Hue University of Medicine and Pharmacy (IEC no-H2020/013).

2.3. Study population

H. pylori strains were isolated from the gastric mucosa of dyspeptic patients who underwent upper endoscopy at Hospital of Hue University of Medicine and Pharmacy from January 2020 to July 2021. Two antral biopsies were obtained. One biopsy was used for the rapid urease test, and if tested positive, the other biopsy was transported in sterile 0.9% NaCl solution to the Microbiology Department of Hospital of Hue University of Medicine and Pharmacy within 2 hours of collection for culture.

2.4. *H. pylori* culture

Biopsy specimens were homogenized and inoculated on Columbia agar plates supplemented with 7% defibrinated sheep blood and selective antibiotics (DENT, Oxoid). The plates were incubated at 37°C under micro-aerobic conditions (GENbox microaer or GENbag microaer; BioMérieux, France) for 7 days. All isolates were presumptively diagnosed as *H. pylori*-positive by colony morphology, microscopic examination, positive biochemical tests of urease, catalase and oxidase, and were finally confirmed as *H. pylori* by PCR assay targeting the *glmM* gene (Table 1) [6].

2.5. Antimicrobial susceptibility testing

The minimum inhibitory concentrations (MICs) of CLR, MTZ, LE, AMX and TE were determined by Epsilometer test (E-test) (bioMérieux, France), performed on Mueller - Hinton agar plates (MUELLER - HINTON agar, Merck) supplemented with 7% sheep blood with bacterial suspension turbidity of 3.0 McFarland and incubated at 37°C under microaerophilic conditions for 72 h. The resistance breakpoints of *H. pylori* are defined as MIC > 0.5 mg/L for CLR, MIC > 1 mg/L for LE, MIC > 8 mg/L for MTZ, MIC > 0.125 mg/L for AMX and MIC > 1 mg/L for TE according to EUCAST guidelines [7]. Primary resistance was commonly defined as resistance in patients with no prior history of *H. pylori* eradication treatment and secondary resistance was in patients with failed *H. pylori* eradication [1].

2.6. DNA extraction

Genomic DNAs of all *H. pylori* isolates were extracted by using a phenol-chloroform method, following the manufacturer's instructions (^{iVA}pDNA Extraction *Kit - Viet A Corp*.). Extracted genomic DNAs were dissolved in 50µl TE buffer and stored at -70^{o}C until use.

2.7. PCR amplification and nucleotide sequencing of the amoxicillin and tetracycline resistance genes

Mutations in PBP1A and the conserved 535 bp region of the *H. pylori* 16S rRNA gene between nucleotide positions 710 and 1245 (numbering according to the rrnA gene of *H. pylori* strain 26695), respectively, conferring AMX and TE resistance in *H. pylori* strains, were detected via PCR, using previously described primers (Table 1) and PCR conditions [4], [8]. PCR-amplified DNA samples were purified using DNA Clean & Concentrator™ - 5 Catalog No. D4004 (Zymo Research, USA). Sequencing of both strands of PCR amplicons was performed at BMR Genomics, Padova, Italy. Sequence data were analyzed using Geneious R11 software.

2.8. PCR amplification of *H. pylori* virulence genes

H. pylori genotypes *cagA*, *cagE*, *cagT*, *vacA* (s1, s2, m1, m2), *iceA*1, *iceA*2 were amplified via PCR, using previously described primers (Table 1) and PCR conditions [9], [10], [11], [12], [13]. The amplified products were identified via electrophoresis on 2% agarose gel. The negative-*cagA H. pylori* strains were further analyzed by PCR using another pair of primers, Luni1-F and R5280-R (Table 1), to determine whether the whole *cag* PAI was deleted. A fragment of 550

bp was amplified when the whole *cag* PAI was absent [14].

2.9. Statistical analysis

IBM SPSS Statistics for Windows, Version 22.0 (Armonk, NY: IBM Corp) was used for statistical analysis. Differences in proportions were evaluated for significance using the chi-square test or Fisher's exact test. P-values < 0.05 indicates a significant difference.

Table 1. Primers used in this study

Amplified gene	Primer	Sequence (5'-3')	PCR product size (bp)	References
*glm*M	GLM MF	GGATAAGCTTTTAGGGGTGTTAGGGG	140	[6]
	GLM MR	GCATTCACAAACTTATCCCCAATC		
pbp1A	PBP1-F	GCATGATCGTTACAGACACG	905	[8]
	PBP1-R	ATCCACGATTTCTTTACGC		
16S rRNA	rrna TET-F	CTGACGCTGATTGCGCGAA	535	[4]
	rrna TET-R	TGGCTCCACTTCGCAGTATT		
cagA (entire repeat)	CAGT-F	ACCCTAGTCGGTAATGGG	Variable, around 500 bp	[9]
	CAGT-R	GCTTTAGCTTCTGAYACYGC		
cag PAI empty site	Luni1-F	CTGACGCTGATTGCGCGAA	550	[14]
	R5280-R	TGGCTCCACTTCGCAGTATT		
cagE	CagE-F	GCGATTGTTATTGTGCTTGTAG	329	[10]
	CagE-R	GAAGTGGTTAAAAAATCAATGCCCC		
cagT	CagT-F	ATGAAAGTGAGAGCAAGTGT	842	[10]
	CagT-R	TCACTTACCACTGAGCAAAC		
vacA s1/s2	VAI-F	ATGGAAATACAACAAACACAC	259/286	[12]
	VAI-R	CTGCTTGAATGCGCCAAAC		
vacA m1/m2	VAG-F	CAATCTGTCCAATCAAGCGAG	567/642	(11)
	VAG-R	GCGTCAAAATAATTCCAAGG		
iceA1	iceA1-F	GTGTTTTTAACCAAAGTATC	247	(13)
	iceA1-R	CTATAGCCASTYTCTTTGCA		
iceA2	iceA2-F	GTTGGGTATATCACAATTTAT	229/334	(13)
	iceA2-R	TTRCCCTATTTTCTAGTAGGT		

3. RESULTS

3.1. Study population

A total of 52 *H. pylori* strains were isolated from 52 patients, including 26 males and 26 females with a mean age of 42.4 years (15–75 years). There was no prior history of *H. pylori* eradication in 8 patients, whereas 44 had haven *H. pylori* treatment failures. Regarding endoscopic findings, 39 patients were diagnosed with gastritis, 11 with PUD and 2 with atrophy gastritis. All *H. pylori* isolates were confirmed as *H. pylori* by PCR assays targeting the *glmM* gene.

3.2. Phenotypic characteristics of *H. pylori* antibiotic resistance

The total resistance rates of *H. pylori* (regardless of *H. pylori* eradication history) to CLR, MTZ, LE and AMX were 90.4% (47/52), 86.5% (45/52), 65.4% (34/52), 40.4% (21/52), respectively. No strain exhibited resistance to TE. The proportion of strains with secondary resistance was significantly higher than those with primary resistance to CLR, LE and double resistance (P <0.05) (Table 2). Resistance to CLR and AMX was significantly higher in females than in males (P <0.05). There was no difference in age and clinical outcome among different antibiotic resistance in *H. pylori* (data not shown).

Table 2. Prevalence of antibiotic resistance of *H. pylori* strains

Resistance	Total (n=52)	Primary resistance (n=8)	Secondary resistance (n=44)	*P*-value
CLR	47 (90.4%)	5 (62.5%)	42 (95.5%)	< 0.05
MTZ	45 (86.5%)	8 (100%)	37 (84.1%)	
LE	34 (65.4%)	1 (12.5%)	33 (75.5%)	< 0.05
AMX	21 (40.4%)	1 (12.5%)	20 (45.5%)	
TE	0%	0%	0%	
No resistance	0%	0%	0%	
Multidrug resistance	46 (88.5%)	6 (75%)	40 (90.9%)	
Single resistance	6 (11.5%)	2 (25%)	4 (9.1%)	
Double resistance	11 (21.2%)	5 (62.5%)	6 (13.6%)	< 0.05
Triple resistance	21 (40.4%)	1 (12.5%)	20 (45.5%)	
Quadruple resistance	14 (26.9%)	0 (0%)	14 (31.8%)	

3.3. Genotypic characteristics of amoxicillin-resistant isolates

After sequencing of *pbp1A* gene of 9 amoxicillin-resistant strains representing 9 different MIC value groups and 1 AMX-susceptible strain which had the lowest MIC (≤ 0.016 mg/L), the deduced amino acid sequences of PBP1A of AMX-resistant strains were aligned and compared with those of the AMX-susceptible strain and of the *H. pylori* reference strain 26695 (GenBank accession number CP026326). Changes in amino acid in PBP1A listed in Table 3 were only detected in the AMX-resistant strains. No statistical significance was observed by analyzing the relationship between mutations and MICs.

Table 3. Amino acid mutations in PBP1A of amoxicillin-resistant *H. pylori* strains

Strain name	MIC (mg/L)	Amino acid position of PBP1A																	
		A369	V374	N400	S431	464-465	D508	V509	M515	S543	T556	T558	N562	G591	A592	T593	G595	595-596	R608
HP01	0.19					E	N	I					Y			G			
HP05	0.25					K												G	
HP11	0.38						N	I			S			_	_	_			
HP22	0.5		L				N	I										G	
HP38	0.75			_		E	N	I	I	R									
HP31	1.5					E												A	
HP51	2		L						I			S	Y			A	S		
HP16	8					E	N		I										H
HP30	256	T			I		N		I							A	S		

Abbreviations: T: threonine, L: leucine, I: isoleucine, E: glutamate, K: Lysine, N: asparagine, R: arginine, S: serine, Y: tyrosine, A: alanine, G: glycine, H: histidine

(Amino acid substitutions were highlighted in blue; amino acid insertions between residues were highlighted in yellow; amino acid deletions were highlighted in red.)

3.4. Genotypic characteristics of tetracycline-resistant isolates

Mutations in the 16S rRNA, especially in the primary binding site of TE to the ribosome responsible for reduced

susceptibility to TE in *H. pylori*, were examined via sequencing of 5 strains that displayed a slight increase in TE MICs (0.25 - 0.75 mg/L) and comparing with the sequence of the *H. pylori* reference strain 26695 (GenBank accession number AE000511.1). Only two isolates with TE MICs of 0.25 and 0.5 mg/L harboured the A926G mutation, which was located right in the primary binding site of TE on the ribosome.

3.5. Genotypes of *H. pylori*

Of the 52 *H. pylori* isolates screened, *cagA, cagE* and *cagT* were found to be present together in 46 isolates (88.5%). These genes were absent in six isolates (11.5%), and in these cases, the *cag* PAI was also absent. There was no significant difference between clinical outcome and the presence of *cagA, cagE* and *cagT.* The *vacA* s- region was determined in 51 out of the 52 isolates (98.1%), and all possessed the s1 type. *Vac*Am1 alone was identified in 27 (51.9%) isolates, *vac*Am2 alone in 21 (40.4%) isolates, while both *vac*Am1 and *vac*Am2 were identified in 4 (7.7%) isolates, indicating mixed infections. Considering strains with a single *vacA* genotype (n=47), two *vacA* genotypes were identified with the predominance of s1m1 allele combination 27/47 (57.4%), followed by s1m2 20/47 (42.6%). The *vacA* genotype and clinical outcome were not associated.

IceA1 was detected in 22 (42.3%) of the 52 isolates examined; *iceA2* was found in 20 isolates (38.5%). Four isolates (7.7%) were positive for both *iceA1* and *iceA2*, and six isolates (11.5%) did not yield any PCR product for *iceA*. The most frequent *iceA* genotype among patients with gastritis was *iceA2* (43.8%), while for patients with PUD, 63% was *iceA1*. There was a significant association between *iceA2* strains and the presence of PUD (p = 0.039).

Seven different genotypic combinations were recognized and had no statistically significant association with clinical outcomes.

The incidence of virulence genes, their association with clinical outcomes and the statistical analyses are shown in Table 4.

Table 4. Distribution of *H. pylori* genotypes in gastroduodenal diseases

Genotype	Endoscopic findings of 52 *H. pylori*-infected patients			Total
	Gastritis (N=39)	PUD[a] (N=11)	Atrophic gastritis (N=2)	
***cagA* (n = 46)**				
***cagA*⁺**	35 (89.7%)	9 (81.8%)	2 (100%)	46 (88.5%)
***cagA*⁻**	4 (10.3%)	2 (18.2%)	0 (0%)	6 (11.5%)
***cagE* (n = 46)**				
***cagE*⁺**	35 (89.7%)	9 (81.8%)	2 (100%)	46 (88.5%)
***cagE*⁻**	4 (10.3%)	2 (18.2%)	0 (0%)	6 (11.5%)
***cagT* (n = 46)**				
***cagT*⁺**	35 (89.7%)	9 (81.8%)	2 (100%)	46 (88.5%)
***cagT*⁻**	4 (10.3%)	2 (18.2%)	0 (0%)	6 (11.5%)
***vacA* s (n = 51)**				51 (98.1%)
s1	39 (100%)	10 (90.9%)	2 (100%)	51 (98.1%)
s2	0 (0%)	0 (0%)	0 (0%)	0 (0%)
***vacA* m (n = 52)**				52 (100%)
m1	21 (53.8%)	5 (45.5%)	1 (50%)	27 (51.9%)
m2	14 (35.9%)	6 (54.5%)	1 (50%)	21 (40.4%)
***vacA* s/m (n = 47)**				47 (90.4%)
s1m1	21 (53.8%)	5 (45.5%)	1 (50%)	27 (51.9%)
s1m2	14 (35.9%)	5 (45.5%)	1 (50%)	20 (38.5%)
iceA				
iceA1	14 (35.9%)	7 (63.6%)	1 (50%)	22 (42.3%)
iceA2	17 (43.6%)	2 (18.2%)	1 (50%)	20 (38.5%)
***iceA*⁻**	4 (10.3%)	2 (18.2%)	0 (0%)	6 (11.5%)

[a]Peptic ulcer disease

4. DISCUSSION

Antibiotic resistance is the main factor responsible for the failure of *H. pylori* eradication therapy. Maastricht Consensus Report recommends that local surveillance of *H. pylori* antibiotic resistance is mandatory to select appropriate eradication regimens according to local resistance patterns [2]. In this study, we determined the antibiotic susceptibility rates to five commonly used antibiotics in the *H. pylori* strains isolated in the central region of Vietnam. Our results highlighted high overall resistance rates (regardless of previous eradication history) to CLR (90.4%), MTZ (86.5%), LE (65.4%) and AMX (40.4%). However, no resistance to TE was identified. Compared with a previous study conducted in 2015 [3], resistance rates to CLR, MTZ, LE, and AMX, as well as multidrug resistance, have trended to further increase in central Vietnam. Noticeably, the proportion of strains with secondary resistance was significantly higher than those with primary resistance to CLR (secondary - 95.5% vs primary - 62.5%), LE (secondary - 75.5% vs primary - 12.5%), indicating that the role of the *H. pylori* treatment failures in the development of CLR and LE resistance in *H. pylori*. Our results agree with a previous study conducted in central Vietnam by Phan et al. [3]. According to the Maastricht V/Florence consensus report, the CLA-based triple regimens should not be widely used as a first-line treatment for *H. pylori* eradication in central Vietnam since the CLA resistance rate exceeds 15% [2]. Our study also showed that LE was unsuitable for treatment in this region as an alternative drug for *H. pylori* eradication. TE can be a key component of *H. pylori* treatment therapy.

This study found that females showed significantly higher CLA and AMX resistance rates than males (100% vs 89.8% and 53.8% vs 26.9%, respectively, $p < 0.05$). The higher CLR resistance rates in females probably reflect that the increased consumption of macrolides may lead to cross-resistance with CLR, such as clindamycin is used as an alternative to MTZ in the treatment of bacterial vaginosis. The reason for the high rates of AMX resistance in females remains unclear; however, considering the paucity of pharmaco-epidemiological data regarding AMX use in Vietnam, it may be speculated that this drug is used disproportionately in females.

Currently, the detailed mechanism underlying AMX resistance in *H. pylori* is unclear. However, mutations in PBP1A, especially mutational changes located in or adjacent to PBP motifs, are considered the predominant cause of AMX resistance in *H. pylori* due to the reduced affinity of PBP1A for this drug [5]. Interestingly, A369T, V374L, S543R, T556S, T558S, N562Y, T593G/A and G595S amino acid substitutions previously reported in analyzed AMX-resistant strains were similarly observed in our study [15], [16]. Since mutations A369T, V374L are located in or adjacent to the conserved SAIK sequence in motif 1 ($SAIK_{368-371}$), and the mutations T556S, T558S, N562Y are in or adjacent to the conserved KTG sequence in motif 3 ($KTG_{555-557}$), these mutations are possible to reduce the binding of AMX to PBP1. One strain carried a single mutation S543R, which was proven to be individually increased resistance in *H. pylori* by natural transformation [15]. Our study also found some substitutions at C-terminus codons (T593G/A, G595S), which were detected in previously analyzed Amx^R strains. However, results of natural transformation showed these mutations were not specific or played a significant role in AMX resistance in the previous reports [15]. Other mutations, including V509I, M515I and the insertion of amino acids (Gly/Ala) at position 595 were also reported in previous studies. However, the role of these substitutions in AMX resistance is not clear because it was present in both Amx^S strains and Amx^R strains [8], [15]. Intriguingly, our data found unreported mutations in PBP1A, including the substitutions S431I, D508N and R608H, the deletion of three consecutive amino acids in positions 591-593 and the deletion of asparagine at amino acid position 400. However, we could not exclude the presence of these mutations in our Amx^S strains because we could not sequence all our Amx^S strains to compare with Amx^R strains.

No amino acid mutations were detected in all nine AMX-resistant strains in our study, which indicates that these detected amino acid mutations in PBP1A were not specific for Amx^R strains. Our results suggest that AMX resistance in *H. pylori* is associated with multiple amino acid changes in PBP1A and tends to increase with an increase in combinations of amino acid mutations in PBP1A, as seen in other papers [5], [15], [16]. Therefore, it is better to avoid using amino acid mutations at a specific position in PBP1A to determine AMX resistance in *H. pylori.*

All 52 *H. pylori* strains included in this study were TE susceptible in the phenotypic drug susceptibility test. In two low-susceptible *H. pylori* isolates with MICs of 0.25 and 0.5 mg/L, an A926G mutation was detected in the 16S rRNA gene. This mutation is located right in an area that contains

the key binding point of the TE molecule to the bacterial ribosome, thus may reduce TE's efficacy. Our observation is consistent with previous reports showing that TE reduced susceptibility in *H. pylori* is conferred by the A926G nucleotide exchange, but with the MICs different to our study [17].

We found out that the majority of studied *H. pylori* isolates (88.5%) possessed *cagA*, *cagE, cagT* genes, and interestingly, isolates that possessed *cagA* carried both *cagE* and *cagT*, and vice versa. Furthermore*, H. pylori* strains negative for *cagA*, *cagE, cagT* genes were confirmed not to carry the complete *cag* PAI by PCR amplification of cagPAI empty-site. This observation is different from a previous study conducted in central Vietnam, in which only 42.9% of the *cag*A-negative strains were true *cag* PAI negative [18]. It can be explained that the whole *cag* PAI in our *cag*A-negative strains was deleted. Meanwhile, there were partially deleted *cag* PAIs in that previous study. The high prevalence of infection with *cagA*-positive *H. pylori* in our study is comparable to the results obtained in the previous study conducted in the same area [18], indicating that central Vietnam is a region with a high prevalence (> 80%) of the *cagA*-positive strains in *H. pylori* infection. There was no association between *cagA, cagE, cagT* genotypes and clinical outcomes.

In this study, all strains of *H. pylori* contain the *vacA* gene, but they vary in terms of *vacA* allelic type. With reference to the *vacA* signal (s) region, the majority of the isolates (51/52, 98.1%) possess the s1 type, whereas no s2 type could be found, and one strain could not be typed. The impossibility of typing the *vacA* signal sequences may be due to a 61-bp insertion in the signal region which was reported by Ito et al., or for unknown reasons [19]. All *H. pylori* strains contain the *vacA* middle sequences. Nearly equal distribution of *vac*Am1 and *vac*Am2 alleles was encountered in this study (m1-51.9%, m2-40.4%). The prevalence of the *vacA* genotypes s1m1was detected in 57.4%, and s1m2 in 42.6%. No cases for *vac*As2m1 and *vac*As2m2 genotypes were detected in this study. We could not find any relationship between *vacA* genotypes and clinical outcomes.

Concerning *iceA* genotype, our results revealed that the distribution of *iceA1* and *iceA2* were comparable (42.3% and 38.5%, respectively), whereas 7.7% were mixed (*iceA1*+*iceA2*). In our study the rate of *iceA2* genotype was also low in patients with PUD, this finding was found in previous studies [20].

In this study we could not find any relationship between the combined genotypes and clinical outcomes, when assessing the combined presence of the above five genes. The relationship could be more easier for drawing any conclusion when the sample size is increased.

5. CONCLUSION

Overall, resistance rates to CLR, MTZ, LE and AMX were high in *H. pylori* from Central Vietnam, except for TE, which serves as a foundation for developing local guidelines for more effective therapeutic strategies. The commonly reported mutations related to AMX resistance and low-level TE resistance were found in our isolates. Our study suggests the genetic analysis of PBP1A is not applicable for determining AMX resistance in *H. pylori,* and MIC measurement should be considered for effective eradication therapy. Neither the single genes nor the combination of genes was significantly helpful in predicting the clinical outcome of *H. pylori* infection in patients in our study.

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