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Antibiotic resistance and heteroresistance in *Helicobacter pylori* isolates from symptomatic Vietnamese children: A prospective multicenter study

Tu Cam Nguyen[1,2] | Giao Kim Ngoc Le[3] | Dao Thi Hong Pham[4] | Bao Van Pham[3] | Loan Thi Hong Nguyen[5] | Thai Hoang Che[6] | Hiep Thanh Nguyen[7] | Dinh Quang Truong[8] | Annie Robert[9] | Patrick Bontems[2,10] | Phuong Ngoc Van Nguyen[6]

[1]Department of Gastroenterology, City Children's Hospital, Ho Chi Minh City, Vietnam

[2]Faculty of Medicine, Université Libre de Bruxelles, Brussels, Belgium

[3]Department of Microbiology and Parasitology, University of Medicine and Pharmacy at Ho Chi Minh City, Ho Chi Minh City, Vietnam

[4]Department of Genetics, University of Science – Vietnam National University Ho Chi Minh City, Ho Chi Minh City, Vietnam

[5]Department of Gastroenterology, Children's Hospital 2, Ho Chi Minh City, Vietnam

[6]Department of Biostatistics and Informatics, Pham Ngoc Thach University of Medicine, Ho Chi Minh City, Vietnam

[7]Faculty of Public Health, Pham Ngoc Thach University of Medicine, Ho Chi Minh City, Vietnam

[8]Department of Surgery, City Children's Hospital, Ho Chi Minh City, Vietnam

[9]Institut de recherche expérimentale et clinique, Pôle d'épidémiologie et Biostatistique, Université catholique de Louvain, Brussels, Belgium

[10]Department of Gastroenterology, Hôpital Universitaire des Enfants Reine Fabiola, Brussels, Belgium

Correspondence
Tu Cam Nguyen, Department of Gastroenterology, City Children's Hospital, Ho Chi Minh City, Vietnam.
Email: ncamtuvn@gmail.com and cam.tu.nguyen@ulb.be

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Abstract

Background: Antibiotic resistance of *Helicobacter pylori (H. pylori)* is increasing worldwide, with geographical variations, impacting the treatment outcomes. This study assessed the antibiotic resistance patterns of *H. pylori* in Vietnamese children.

Materials and Methods: Symptomatic children undergoing gastroduodenoscopy at two tertiary Children's Hospitals in Ho Chi Minh City were recruited. Antral and corpus biopsies were obtained and cultured separately. Susceptibility to amoxicillin (AMO), clarithromycin (CLA), metronidazole (MET), levofloxacin (LEV), and tetracycline (TET) was determined using E-test. Polymerase chain reaction was performed on another antral biopsy to detect the urease gene, cytotoxin-associated gene A (*cagA*), vacuolating cytotoxin A (*vacA*) genotypes, and 23S rRNA mutations conferring CLA resistance.

Results: Among 123 enrolled children, a high primary resistance rate was found for CLA (68.5%, 61/89), followed by LEV (55.1%), MET (31.5%), AMO (25.8%), and TET (1.1%). Secondary resistance rates were 82.1% (7/28), 71.4%, 53.6%, and 3.6% for CLA, LEV, MET, and TET, respectively. Multidrug resistance was frequent (67.7%), with common patterns including CLA+LEV (20.3%) and CLA+MTZ+LEV (15.2%). Heteroresistance was detected in eight children (6.5%). The A2143G mutation was detected in 97.5% (119/122) of children. 86.1% of children had positive *cagA* strains

Tu Cam Nguyen and Phuong Ngoc Van Nguyen should be considered the joint first authors.

and 27.9% had multiple *vacA* genotypes. No factor was significantly associated with antibiotic resistance.

Conclusions: The alarming rate of antibiotic resistance for *H. pylori*, especially for CLA, with emerging multi- and hetero-resistant strains, pose a major treatment challenge that precludes CLA use as empirical therapy. Biopsies from both antrum and corpus can improve *H. pylori* culture, allowing tailored treatment based on antimicrobial susceptibility.

KEYWORDS
23S rRNA point mutations, antibiotic resistance, *Helicobacter pylori*, heteroresistance, symptomatic Vietnamese children

1 | INTRODUCTION

Helicobacter pylori (*H. pylori*), a Gram-negative bacterium that colonizes the stomach, is a major pathogen linked to gastritis, peptic ulcers, and even gastric cancer.[1] Its prevalence ranges from 13.4% to 84.2% in adults and 1.7%–97.1% in children across countries. The infection rates are lowest in Northern America and highest in certain regions of Latin America, Asia, and Africa.[2] *H. pylori* eradication therapy prevents complications and gastric cancer development.[3] But the emergence and spread of antibiotic-resistant strains have become a global concern.[4] Along with the prevalence of *H. pylori*, the antibiotic resistance rates also vary notably across regions.[5]

Additionally, quinolones and tetracycline are contraindicated in children, limiting eradication options. Increasing resistance to commonly used antibiotics, especially clarithromycin and metronidazole, has significantly reduced empiric eradication effectiveness. The pooled prevalence of resistance in children ranged from 10% to 85% for clarithromycin, 20% to 81% for metronidazole, and 4% to 29% for levofloxacin.[6] Heteroresistance, when susceptible and resistant isolates coexist in the same patient, has also emerged, leading to additional difficulties in detecting resistance and in choosing between treatment options.

Recent studies showed that infection with cytotoxin-associated gene A (*cagA*)-positive and vacuolating cytotoxin A (*vacA*) *s1m1 H. pylori* strains was associated with higher resistance against metronidazole, amoxicillin, and levofloxacin in Western countries while *vacA s2m2* strains tend to show less antibiotic resistance.[7] Additionally, several point mutations in the *H. pylori* gene have been found to be associated with antibiotic resistance.[8–10] As one of the most commonly used macrolides in children, clarithromycin resistance has been associated with point mutations in the domain V of 23s rRNA gene.[8] Therefore, individualized eradication treatment can be based on antimicrobial susceptibility testing obtained either by culture-based methods or molecular biology methods that detect point mutations conferring antibiotic resistance in order to improve the eradication efficacy.[4,11]

In Vietnam, *H. pylori* prevalence is estimated at 70.3%.[12] It ranges from 34% to 88% in children.[13–15] Previous single-center studies have shown an increasing trend of *H. pylori* gastritis and peptic ulcer disease in children.[16–18] Data on antibiotic resistance and relevant mutations in the 23S rRNA gene in Vietnamese children need to be updated and more comprehensive. Given the significant health implications, a prospective multicenter study would be useful to explore the antibiotic resistance patterns and point mutations in 23S rRNA of *H. pylori* in Vietnamese children.

2 | MATERIALS AND METHODS

2.1 | Study population and design

From October 2019 to May 2021, we prospectively recruited all consecutive symptomatic children aged 4–16 years undergoing upper gastrointestinal (GI) endoscopy at two tertiary children's hospitals in Ho Chi Minh City (HCMC); City Children's Hospital (located in the rural Binh Chanh District) and Children's Hospital 2 (located in the urban District 1). These hospitals provide specialized pediatric services to children from HCMC and surrounding provinces of Vietnam.

Written informed consent was obtained from parents/legal guardians of all participants, with informed assent also signed by children older than 12 years. The study was approved by the Scientific Council of the Pham Ngoc Thach University of Medicine (N°2683/QĐ-TĐHYKPNT) and the local Ethics Committees of both hospitals (N° 37/QĐ-BVNĐTP). Demographic characteristics, *H. pylori* treatment history, anthropometric measurements, and endoscopic findings were collected. Nutritional status was classified according to the World Health Organization Child Growth Standards Chart. Endoscopic lesions were described according to Minimal Standard Terminology for Digestive Endoscopy.[19]

In each patient, two biopsies (one from the antrum and one from the corpus) were taken for *H. pylori* culture. Two additional biopsies (one from the antrum and one from the corpus) were obtained for histopathology. The updated Sydney classification system was used for histological diagnosis.[20] A final antral biopsy was used for a rapid urease test (RUT), followed by a polymerase chain reaction (PCR) assay for urease gene-*ureA*. PCR urease-positive samples were further detected for virulence factors (cytotoxin-associated gene A (*cagA*), vacuolating cytotoxin A (*vacA*)), and point mutations of clarithromycin resistance in the 23S rRNA gene.

Inclusion criteria were children having a positive *H. pylori* culture together with antimicrobial susceptibility testing results. Exclusion criteria were children referred for interventional endoscopy, and those treated with proton pump inhibitors (PPIs), histamine receptor

blockers, antacids, bismuth salts within 2 weeks, or antibiotics within 4 weeks before endoscopy.

2.2 | *H. pylori* culture and antimicrobial susceptibility testing

The two antrum- and corpus biopsies were stored separately at 4°C in the transport mediums with brain heart infusion broth with 1% agar, and transferred to the microbiology laboratory within 4 h. After processing, samples were plated onto special media with trypticase agar, 5% sheep blood, 20% fetal bovine serum, 1% IsoVitaleX™, and an antibiotic mixture, and incubated microaerophilically at 35°C for up to 14 days. Identification of *H. pylori* was made by positive urease, catalase, and oxidase tests after incubation.

H. pylori strains were assessed for antimicrobial susceptibility to five antibiotics (amoxicillin (AMO), clarithromycin (CLA), metronidazole (MET), levofloxacin (LEV), and tetracycline (TET)) using E-test method (bioMérieux, Belgium). The minimum inhibitory concentrations (MICs) were classified as susceptible, intermediate, and resistant based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST Version 13.0) guidelines. The resistance break points were >0.125 mg/L for AMO, >0.25 mg/L for CLA, >8 mg/L for MET, >1 mg/L for LEV, and >1 mg/L for TET. High levels of antibiotic resistance were defined as MICs >8 mg/L for AMO and CLA, >32 mg/L for MET, and ≥ 32 mg/L for LEV.[21] In patients with two available antibiograms in both antrum and corpus, antibiotic susceptibility was considered resistant if at least one site showed resistance.

2.3 | PCR analysis for detection of *H. pylori* urease gene, *cagA*, and *vacA* genotypes

Genomic DNA was extracted from residual gastric biopsies after rapid urease testing (Urease NS, Vieta Corporation, Vietnam). *H. pylori* urease gene was detected by Real-time PCR using AccuPid *H. pylori* Detection Kit (KT Biotech, Vietnam).

Detection of genotypes of *cagA* and *vacA* genes was based on Multiplex PCR using AccuLite *H. pylori* Genotyping Kit (KT Biotech, Vietnam) with the primer sequences referenced in the previous Vietnamese study as reported by Trung et al.[22]

2.4 | Detection of point mutations in the 23s rRNA gene of *H. pylori*

The point mutations of *H. pylori* 23S rRNA were detected via PCR amplification of the domain V of the 23S rRNA gene, followed by Sanger sequencing on ABI 3130 Applied Biosystem® using the Big Dye Terminator methodology (Applied Biosystems®), referenced by Matta et al.[23] The reference sequence for the 23S rRNA region in CLA-susceptible *H. pylori* strain 26,695 (NC_000915), was used to identify mutations associated with CLA resistance in clinical strains. The Sanger sequencing results for the 23S rRNA regions were aligned and compared with the reference sequences using the Geneious tool (version 2019.2.3). Mutations that occurred at positions 2142 and 2143 of the 23S rRNA sequence region in clinical strains (including A2142G, A2142C, and A2143G) were recorded.

2.5 | Criteria for *H. pylori* infection and definition of antibiotic resistance

According to ESPGHAN/NASPGHAN criteria, *H. pylori* infection was confirmed by a positive culture or histological evidence in combination with either a positive RUT or PCR assay of *ureA*.[11] *H. pylori* status was defined as doubtful if histology alone was positive, or if histology was negative but RUT was positive and/or PCR assay of *ureA* was positive. *H. pylori* status was negative when all four biopsy-based tests were negative.

Primary resistance was defined as resistance observed in patients who had not previously received treatment for *H. pylori* infection, commonly referred to as naive patients. By contrast, secondary resistance was defined as the presence of resistance in patients with a previous history of *H. pylori* treatment, referred to as non-naive patients. Antibiotic resistance patterns were classified as mono, double, triple, quadruple, and quintuple resistance. Multidrug resistance was defined as resistance to two or more antibiotics. Heteroresistance was considered as the coexistence of susceptible and resistant isolates to the same antibiotic at different biopsy sites of the stomach in the same patient.[24]

2.6 | Statistical analysis

All statistical analyses were performed using Stata SE 17.0 (Stata Corporation, Texas, USA). Differences in antibiotic resistance rates among categorical variables (gender, living area, history of *H. pylori* therapy, and endoscopic lesions) were evaluated by the chi-square test (or Fisher's exact test), and by Cochran–Armitage trend test for ordered categorical variables (age group and nutritional status). Agreement between antibiotic resistance rates in two biopsy sites was evaluated using Cohen's kappa coefficient. Wilcoxon rank-sum test was used to compare MICs values between the two biopsy sites because MICs values had non-Gaussian distributions. Factors were tested for association with *H. pylori* antibiotic using simple logistic regression, then included in a multiple logistic regression if univariate p-value <0.25. All tests were two-sided and the statistical significance level was set to 0.05.

3 | RESULTS

3.1 | Patient characteristics

A total of 394 children underwent upper GI endoscopy at two children's hospitals during the study period. *H. pylori* status was positive in 306/394 patients (77.7%), doubtful in 40/394 (10.1%), and

negative in 48/394 (12.2%). Out of 40 patients with doubtful infections, six patients had only positive *H. pylori* in histology, 15 had only a positive RUT, and 19 had both a positive RUT and a positive PCR assay of the *ureA* gene. Among 306 infected ones, only 150 had positive culture results, yielding a sensitivity of 49%. Due to low bacterial growth, antibiotic susceptibility testing results were obtained for 123 patients and included in the study. Among these 123 children (mean age: 9.5 ± 2.5 years, 54.5% girls), peptic ulcers were observed in 26% (32/123). Out of 117 children with available information on *H. pylori* treatment history, 23.9% (28/117) received prior treatment. Demographic characteristics, nutritional status, endoscopy findings, and history of *H. pylori* treatment were similar between the 123 patients with antibiogram results and 183 *H. pylori*-infected patients without antibiogram (Table 1).

Regarding virulence factors, 105/122 (86.1%) children (one missing data) were positive with *cagA* gene. The *vacA* genotypes were *s1m1* (48/122, 39.3%), *s1m2* (33/122, 27.1%), *s1/m1m2* (30/122, 24.6%), *s1s2/m2* (3/122, 2.5%), *s2/m1m2* (1/122, 0.8%), and *s1* (7/122, 5.7%) (incomplete *VacA*).

3.2 | Primary and secondary *H. pylori* antibiotic resistance rates

Overall, 79.7% (98/123) of *H. pylori* strains were resistant to at least one antibiotic. The overall resistance rates to AMO, CLA, MET, LEV, and TET were 25.2%, 72.4%, 38.2%, 60.2%, and 1.6%, respectively.

Among the 89 naive patients, the primary resistance rate was the highest for CLA (68.5%, 61/89), followed by LEV (55.1%, 49/89), MET (31.5%, 28/89), AMO (25.8%, 23/89), and TET (1.1%, 1/89).

Among the 28 non-naive patients, the secondary resistance was similar in AMO (25%, 7/28) but was higher in CLA (82.1%, 23/28), LEV (71.4%, 20/28), MET (53.6%, 15/28), and TET (3.6%, 1/28).

There were no statistically significant differences in the rates of resistance to CLA, LEV, AMO, and TET between naive- and non-naive patients. However, for MET specifically, the resistance rate differed significantly between the two groups. Indeed, the resistance rate of MET was higher in non-naive patients with an OR of 2.51 (95% CI: 1.06–5.98, $p = 0.04$). Additionally, while 24.7% (22/89) of strains isolated from naive patients were susceptible to all antibiotics, this rate was only 7.1% (2/28) from non-naive patients (OR = 4.23, (95% CI: 1.05–28.3), $p = 0.04$). No association was found between age groups, gender, living area, nutritional status, peptic ulcers, *cagA*, *vacA* genotypes, and resistance rate of each antibiotic.

3.3 | MICs profiles of antibiotics

The distribution of MICs values for five antibiotics in *H. pylori* isolates in two biopsy sites is shown in Figure 1. The median MICs were similar between the antrum and the corpus. The majority of *H. pylori* strains had MICs at the resistance threshold (0.125 mg/L) for AMO, with a small percentage showing very high MICs (>8 mg/L) (4/111 (3.6%) in the antrum and 3/98 (3.0%) in the corpus). By contrast, about 20% of strains had high MICs values at 8 mg/L for CLA and > 32 mg/L for MET. Approximately 50% of *H. pylori* strains had resistant MICs values of 32 mg/L for LEV. Finally, both resistant-*H. pylori* strains had a MIC of 4 mg/L for TET.

3.4 | Multidrug antibiotic resistance

Among *H. pylori* isolates, 13% (16/123) showed monoresistance while 67.7% (82/123) exhibited multidrug resistance. Of these, 27.6% (34/123) had double resistance, with CLA + LEV resistance being the most common (20.3%, 25/123). Triple resistance was observed in 26.4% (33/123), with CLA + MET+LEV resistance being predominant (15.2%, 19/123). Quadruple resistance was found in 12.2% (15/123), and no strains were resistant to all five tested antibiotics. No significant differences were observed between naive- and non-naive patients in terms of frequency of monoresistance, double resistance, triple resistance, quadruple resistance, or quintuple resistance (Table 2).

3.5 | Antibiotic heteroresistance

Among the 123 patients studied, 69.9% (86/123) had antibiograms from two gastric sites, 20.3% (25/123) had antibiograms only from the antrum and 9.8% (12/123) had antibiograms only from the corpus. The concordance of antibiotic resistance rates between antrum and corpus was high, with agreement ranging from 96.5% to 100% and kappa coefficient values from 92% to 100%. However, 6.5% (8/123) of patients presented heteroresistance. Heteroresistance to AMO and LEV was observed in both naive and non-naive patients, whereas heteroresistance to MET was found in three naive patients only. Notably, one non-naive patient exhibited concurrent heteroresistance to both CLA and LEV. Details on the characteristics of patients with antibiotic heteroresistance of *H. pylori* are presented in Table 3.

3.6 | Point mutations in the 23S rRNA gene conferring CLA resistance

Among 123 children with MICs values of CLA, 122 children performed DNA sequencing of the 23S rRNA for point mutations associated with CLA resistance, while one had missing data. The A2143G mutation on the *H. pylori* gene was detected in 97.5% (119/122) of children. Neither A2142G nor A2142C mutation was found. The A2143G mutation was detected in 97.7% (86/88) of CLA-resistant strains and 97.1% (33/34) of CLA-susceptible strains.

Regarding *vacA* genotypes of patients with CLA-susceptible strains, we observed the following *vacA* genotypes: 29.4% (10/34)

TABLE 1 Characteristics of the *H. pylori*-infected patients with and without antimicrobial susceptibility testing results.

Patient characteristics	Children with AST $N=123$	Children without AST $N=183$	p
Age (mean age ± SD)	9.5 ± 2.5	9.4 ± 2.5	
Age group			
< 6 years	7 (5.7)	12 (6.6)	0.37
6–<11 years	86 (69.9)	119 (65.0)	
≥11 years	30 (24.4)	52 (28.4)	
Gender			
Boys	56 (45.5)	87 (47.5)	0.66
Girls	67 (54.5)	96 (52.5)	
Living area			
Provinces	56 (45.5)	107 (58.5)	0.22
HCMC	67 (54.5)	76 (41,5)	
Rural	42 (34.2)	47 (25.7)	
Urban	25 (20.3)	29 (15.8)	
Nutritional status			
Malnutrition	21 (17.1)	33 (18.0)	0.13
Normal weight	58 (47.2)	101 (55.2)	
Obesity/Overweight	44 (35.8)	49 (26.8)	
Endoscopic findings			
Ulcers	32 (26.0)	36 (19.7)	0.19
Nonulcers	91 (74.0)	147 (80.3)	
History of *H. pylori* treatment ($n=297$)	$n=117$	$n=180$	0.89
Yes	28 (23.9)	41 (22.8)	
No	89 (76.1)	139 (77.2)	

Abbreviations: AST, antimicrobial susceptibility testing; HCMC, Ho Chi Minh City.

Note: Data are numbers and percentages unless stated.

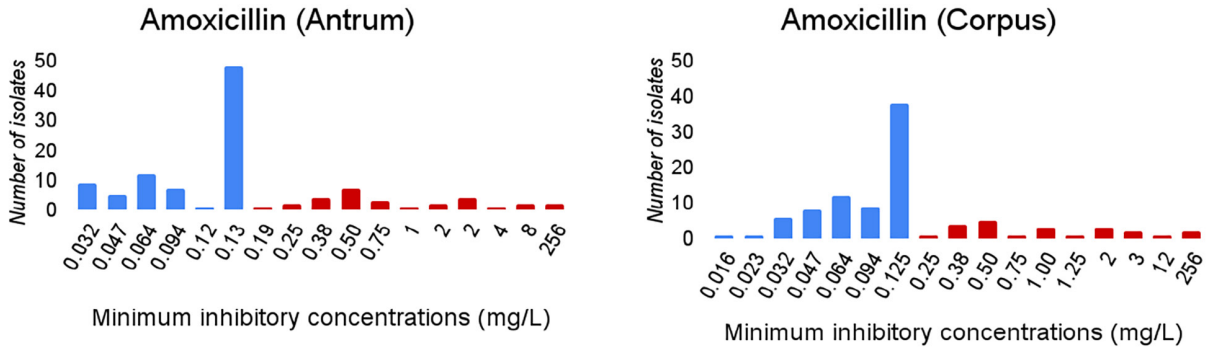
s1/m1m2, 5.9% (2/34) *s1s2/m2*, 2.9% (1/34) *s2/m1m2*, 35.3% (12/34) *s1m1*, 14.7% (5/34), and 11.8% (7/34) incomplete *vacA*.

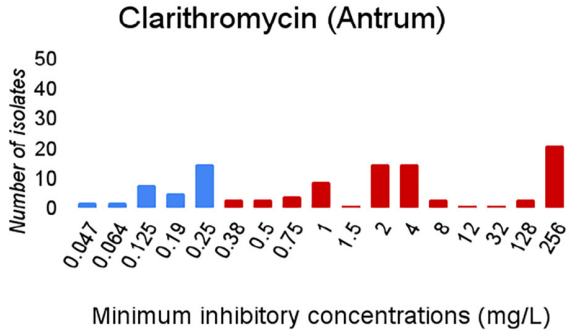
4 | DISCUSSION

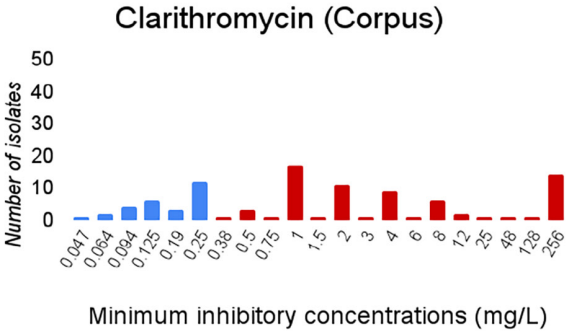
H. pylori infection remains an important health issue among children in HCMC, with a high prevalence (87.7%)[15,25] and a high proportion of peptic ulcer disease.[18,26] Successful eradication of *H. pylori* is crucial to prevent complications and the recurrence of ulcers. *H. pylori* culture testing, as the gold standard for diagnosis, is recommended during GI endoscopy to gather data on resistance rates in the population and to tailor the treatment according to antimicrobial susceptibility.[11,27] Our data showed alarming resistance rates to the commonly used antibiotics for *H. pylori* treatment in symptomatic Vietnamese children, aligned with those observed in Vietnamese adults.[28,29] Located in a tropical region, Vietnam is characterized by a high rate of infectious diseases and consequently high antibiotic consumption.[30] Additionally, antibiotics can be purchased without a prescription.[31] Depending on the patient's antibiotic exposure related to the epidemiology of infectious disease and antibiotic stewardship, the resistance rate can vary between regions.

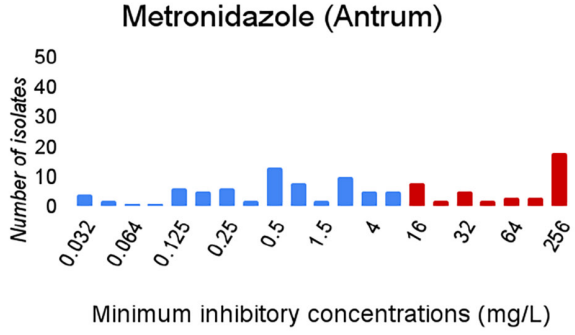
CLA has been commonly used in first-line *H. pylori* treatment regimens.[16,32] In Vietnam, CLA resistance has been increasing rapidly, with resistance rates ranging from 33% to 66.1% for primary resistance,[28,32,33] and 43.6% to 94.3% for secondary resistance in adults[28,29,32,34,35] since the first report with a low prevalence (<20%) in 10 years ago.[36] Our study showed a high resistance rate to CLA (primary: 68.5%; secondary: 82.1%), which is consistent with more recent studies in Vietnamese children. In the Mekong Delta region (comprising 13 provinces located directly to the west of HCMC), an overall resistance rate in children was reported at 80.6% (primary: 77%, secondary: 92.6%).[18] In Hanoi (Northern Vietnam), the CLA resistance rate was extremely high at 96.7% in children with peptic ulcers, regardless of the pretreatment history.[37] However, our CLA resistance rate is approximately 2.5–3.5 times higher than reported for America (19%; 95% CI:13–26), Europe (24%; 95% CI: 19–30), and Southeast Asia (29%; 95% CI: 22–38), but similar to that in the Western Pacific region (85%; 95% CI: 80–90).[6] This may be related to CLA exposure during childhood, including the treatment of respiratory infections, self-medication with antibiotics,[31] over-the-counter purchase, and inadequate adherence to treatment regimens as CLA is an antibiotic in the first-line therapy for *H. pylori*.

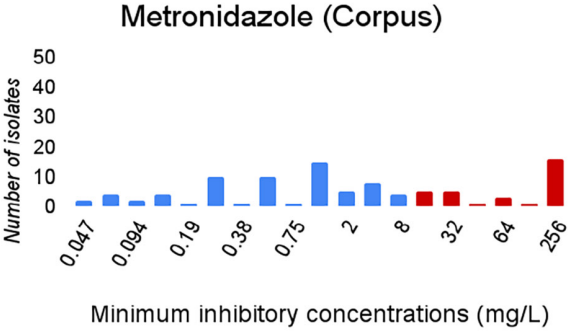
Additionally, several point mutations in the 23s rRNA gene of *H. pylori* are associated with CLA resistance.[38–40] Through Sanger

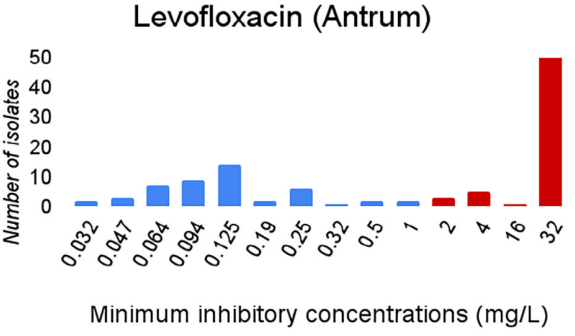


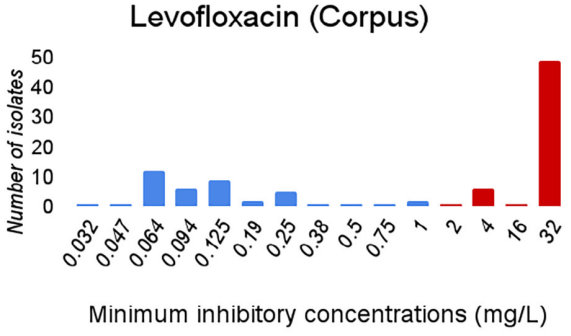


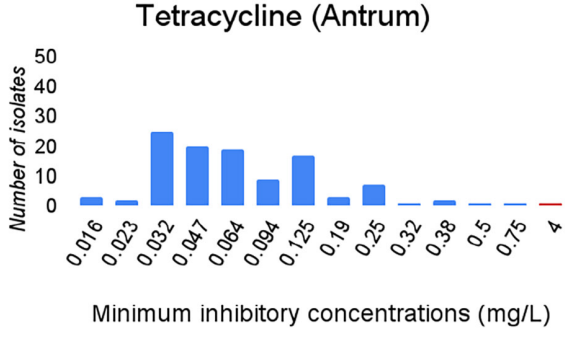












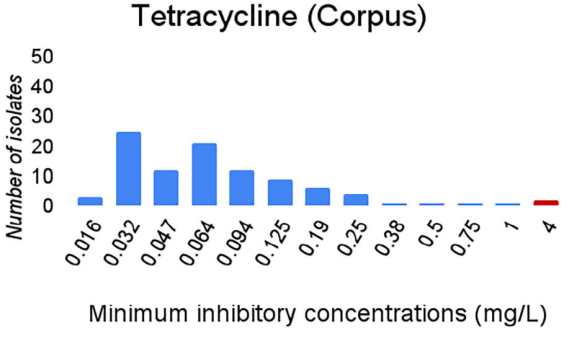


FIGURE 1 Minimum inhibitory concentrations distribution of five antibiotics to *H. pylori* isolates in the antrum ($n = 111$) and corpus ($n = 98$). The resistance cutoff values for each antibiotic were >0.125 mg/L for amoxicillin, >0.5 mg/L for clarithromycin, >8 mg/L for metronidazole, and >1 mg/L for both levofloxacin and tetracycline. The blue and red bars represent susceptible and resistant strains, respectively.

TABLE 2 Antibiotic resistance patterns of *H. pylori* isolates.

Resistance patterns	All Patients[a] N = 123	Naïve patients N = 89	Non-naive patients N = 28	OR (95% CI)	p
Fully susceptible—No (%)	25 (20.3)	22 (24.7)	2 (7.1)	4.23 (1.05–28.3)	0.04
Monoresistance —No (%)	16 (13.0)	11 (12.4)	5 (17.9)	1.54 (0.49–4.89)	0.46
CLA	9 (7.3)	7 (7.9)	2 (7.1)	0.90 (0.18–4.61)	0.90
MET	3 (2.4)	2 (2.3)	1 (3.6)	1.61 (0.14–18.5)	0.71
LEV	4 (3.3)	2 (2.3)	2 (7.1)	3.35 (0.45–24.9)	0.24
Double resistance—No (%)	34 (27.6)	26 (29.2)	7 (25.0)	0.81 (0.31–2.13)	0.67
AMO-CLA	3 (2.4)	3 (3.4)	0 (0.0)	NA	
CLA-MET	6 (4.9)	3 (3.4)	3 (10.7)	3.44 (0.65–18.1)	0.15
CLA-LEV	25 (20.3)	20 (22.5)	4 (14.3)	0.58 (0.18–1.85)	0.35
Triple resistance—No (%)	33 (26.4)	21 (23.6)	9 (32.1)	1.53 (0.60–3.89)	0.37
AMO-CLA-MET	3 (2.4)	3 (3.4)	0 (0.0)	NA	
AMO-CLA-LEV	9 (7.3)	6 (6.7)	3 (10.7)	1.67 (0.39–7.12)	0.49
AMO-MET-LEV	1 (0.8)	1 (1.1)	0 (0.0)	NA	
AMO-LEV-TET	1 (0.8)	1 (1.1)	0 (0.0)	NA	
CLA-MET-LEV	19 (15.2)	10 (11.2)	6 (21.4)	2.15 (0.71–6.58)	0.18
Quadruple resistance—No (%)	15 (12.2)	9 (10.1)	5 (17.9)	1.93 (0.59–6.34)	0.28
AMO-CLA-MET-LEV	14 (11.4)	9 (10.1)	4 (14.3)	1.48 (0.42–5.24)	0.54
CLA-MET-LEV-TET	1 (0.8)	0 (0.0)	1 (3.6)	NA	

Abbreviations: AMO, amoxicillin; CLA, clarithromycin; MET, metronidazole; LEV, levofloxacin; TET, tetracycline.

Note: NA denotes not available.

[a]Missing history of previous treatment for *H. pylori* in six patients.

sequencing, we identified a high rate (98.4%) of a unique mutation at the A2143G nucleotide position. This mutation is shown to confer high-level resistance to CLA[8,41] and is associated with a higher risk of treatment failure.[42,43] This is the most common point mutation of 23S RNA gene of *H. pylori* in many regions worldwide, and Vietnamese adults (40.5%).[44] No related studies have been conducted on Vietnamese children.

Surprisingly, this mutation was detected in patients with CLA-resistant (97.7%) and CLA-susceptible (97.1%) *H. pylori* based on culture. This could be explained by the mixed infections and false positive results of the PCR. Some patients may be infected with multiple strains including both CLA-resistant and susceptible *H. pylori* strains, called mixed infections or heteroresistant infections. In our study, the *H. pylori* culture and mutation analysis were performed on separate biopsies. The culture results showed that there was an antibiotic heteroresistance rate of 6.5% which implies mixed infections. The *vacA* genotyping results also demonstrated that 27.9% of patients had multiple *vacA* genotypes in the same gastric biopsy (*s1/m1m2*, *s1s2/m2*, and *s2/m1m2*), suggesting multiple strains infections. The coexistence of multiple *H. pylori* strains in the same patient may be the result of simultaneous colonization, which has been described in many studies,[45,46] including in the Vietnamese population.[47] In addition, PCR-based methods are highly sensitive but fail to differentiate between alive and dead bacteria, which can lead to false positive results. As *H. pylori* culture has a lower sensitivity, this high prevalence of A2143G point mutation may indicate that CLA resistance might be higher than observed by E-test in Vietnamese children. Consequently, CLA may rarely be effective in eradication regimens in our region, with implications for clinical practice. Based on the latest consensus on *H. pylori* management in Vietnamese adults (2023)[48] and in children (2017),[49] CLA-based regimens are no longer recommended in empiric treatment unless the AST shows a susceptible result.

Resistance to MET is also a growing concern, particularly in children. Our data revealed high resistance rates of MET (overall: 38.2%, primary: 31.5%, and secondary: 53.6%), which aligns with findings in children from Hanoi (30.5%),[37] and Mekong Delta (overall: 49.4%, primary: 51.4%, and secondary: 42.6%).[18] However, these rates were still lower than those reported in Vietnamese adults, ranging from 66.2% to 76.1%[29,33,34] as well as in Belgium (52%),[50]

TABLE 3 Characteristics of patients with heteroresistance.

Patient No.	Age (years)	Gender	History of *H. pylori* treatment	Antibiotics	Antrum		Corpus	
					Antibiotic susceptibility	MICs (mg/L)	Antibiotic susceptibility	MICs (mg/L)
1	8.1	Female	Yes	Amoxicillin	Resistant	0.25	Susceptible	0.125
2	4.5	Male	No	Amoxicillin	Susceptible	0.125	Resistant	1.25
3	11.0	Female	No	Metronidazole	Susceptible	1.5	Resistant	256
4	7.5	Female	No	Metronidazole	Resistant	128	Susceptible	8
5	8.6	Male	No	Metronidazole	Susceptible	1.5	Resistant	256
6	10.7	Female	No	Levofloxacin	Susceptible	0.125	Resistant	4
7	9.0	Male	Yes	Levofloxacin	Susceptible	0.125	Resistant	32
8	10.2	Female	Yes	Levofloxacin	Susceptible	0.094	Resistant	32
				Clarithromycin	Susceptible	0.19	Resistant	2

Abbreviation: MICs, minimum inhibitory concentrations.

Germany (59%),[51] Iran (71%),[52] and China (81.7%).[53] The difference in resistance rates may be related to varying levels of MET exposure in different regions, as a history of MET use increases the risk of resistance.[54] MET is not commonly used in children as its indications are limited to giardiasis, bacterial dysentery, or anaerobic respiratory tract infections. Alternatively, MET resistance can be overcome in vivo by using higher dosages (30 mg/kg/day).[55] Nevertheless, MET-based regimens are currently recommended as first-line therapy in Vietnam due to the CLA resistance rates exceeding 20%.[4,11,48] This may contribute to the further development of MET resistance.

The overall LEV resistance rate in our study was the second highest (60.2%, primary: 55.1%, secondary: 71.4%), surpassing MET but lower than CLA. This rate is similar to that found in Vietnamese adults (67.5%),[56] but considerably higher than rates reported in Europe (4%),[6] China (18.8%–22.8%),[53,57] and the Eastern Mediterranean region (29%).[6] LEV resistance rates in children in Vietnam showed varying rates, with 9.9% in Hanoi,[37] and 45.1% in Mekong Delta.[18] This discrepancy may be due to differences in LEV consumption across communities based on local antibiotic stewardship. In some regions with high rates of macrolides resistance, LEV is commonly prescribed to treat other common infectious conditions such as acute bacterial sinusitis, bacterial bronchitis, and atypical pneumonia in both adults and children.[30,58] Furthermore, the integration of LEV as an empiric alternative first-line treatment for *H. pylori* in Vietnamese adults has also contributed to the emergence and rapid increase of LEV resistance.[48] Importantly, self-medication is also very common in Vietnam.[31] These factors might contribute to the high level of LEV resistance in children.

Although the prevalence of AMO resistance is generally low worldwide,[5,59,60] our study revealed the AMO resistance rates were moderately high, aligned with that observed in Vietnamese adults.[61] In 2022, Tran et al found a primary resistance rate of 25.7% for AMO on 101 *H. pylori* isolates and its association with pbp1A point mutation in HCMC.[61] However, compared to other studies conducted in children, our results are still lower than rates reported in other provinces such as Hanoi (88.7%)[37] and Mekong Delta (71.7%),[18] as well as in Egypt (95%),[62] and African regions (72.6%).[63] AMO resistance rates vary across different geographical regions due to the differences in antimicrobial testing methods, antibiotic consumption, and exposure levels. It is important to note that AMO resistance rates have increased over the past decade.[64] AMO, particularly in combination with clavulanate, is commonly used to treat respiratory tract, skin, and urinary tract infections in children. However, a few strains exhibit very high MICs for AMO, suggesting that higher doses may be effective in overcoming the resistance in therapeutic regimens.[65] Recent research in Vietnamese adults has suggested that circulating *H. pylori* strains carry a mutant gene that confers resistance to AMO.[61] These factors probably contribute to the observed high resistance of *H. pylori* to AMO in Vietnamese children.

In our study, resistance to TET remained at a low level (<2%), as in other studies in Europe or America.[5] However, a study in the Mekong Delta reported a higher primary resistance rate of 10.9%,[18] while no resistance was found in a study in Hanoi.[37] Currently, TET should not be given to children because of adverse effects on bones and teeth. The TET-resistant strains in children are probably transmitted from adults, where resistance rates ranged from 5.8%[33] to 10.9%,[66] since TET is commonly used as a first-line regimen in adults.[48]

In Vietnam, most studies have reported higher secondary resistance compared with primary resistance.[28,29,44] Our results showed similar trends for CLA, LEV, and TET, but without statistically significant differences. Particularly for MET, the resistance rate in non-naive patients was found to be twice as high as that of naive patients. This may be because MET, despite its limited indications in children, is commonly used in first-line regimens for *H. pylori* treatment, resulting in a higher MET resistance rate in the non-naive group due to previous exposure to this antibiotic. Meanwhile, the remaining antibiotics are more commonly prescribed for respiratory infections in children, leading to similar rates of resistance between the naive- and non-naive groups.

In terms of multidrug resistance, we found that the resistance rate to at least two antibiotics was very high among *H. pylori* strains

(67.7%). The main resistance patterns were CLA + LEV (20.3%), CLA + MTZ + LEV (15.2%), and AMO + CLA + MTZ + LEV (11.4%). In most regions, double resistance to CLA + MET was the most commonly observed pattern.[6] However, in Vietnam, our results differed from those reported in Hanoi, where resistance to AMO + CLA was predominant (55%), followed by AMO + CLA + MET (23.8%). Recent research on Vietnamese adults in Thai Binh Province revealed a double resistance rate of 42.5% to AMO + CLA.[56] The multidrug resistance patterns vary by geographic region and probably depend on the level of antibiotic consumption and commonly used antibiotics. This multiresistance issue complicates the treatment of *H. pylori* in a highly prevalent area like Vietnam.

Heteroresistance in *H. pylori* refers to the coexistence of susceptible and resistant strains of the same antibiotic in the same patient. This could be mixed infections or the coexistence of susceptible and resistant variants of the same strain. Heteroresistance can occur within the same gastric biopsy site (intra-niche) or in different biopsy sites (inter-niche).[45,67,68] It can happen spontaneously or as a result of antibiotic exposure.[69] In this study, we detected inter-niche heteroresistance in 6.5% of cases as we performed *H. pylori* culture from two biopsy sites. Missing the resistant strains or variants during antimicrobial susceptibility testing in patients may lead to eradication failure.

The first limitation of our study is that the number of participants was lower than expected due to difficulties in recruiting endoscopic patients during the Covid-19 pandemic period. Nevertheless, the observation rate was greater than 0.50 used for sample size detection. Second, *H. pylori* isolates were not accessible in children with severe GI bleeding due to peptic ulcers because these patients usually require urgent interventional endoscopic therapy and PPIs treatment.

Our study has, however, key strengths. Firstly, a routine *H. pylori* culture was performed on all symptomatic children undergoing upper GI endoscopy, with sampling from two biopsy sites, enhancing the robustness of our findings. Secondly, excluding patients who received PPIs and/or antibiotics before endoscopy optimized the reliability of biopsy-based tests. Moreover, we detected the point mutations of 23 s rRNA conferring the CLA resistance in *H. pylori*, which has not been reported in other studies in Vietnamese children adding novel insights to the field. Therefore, these data, obtained from two high-volume tertiary children's hospitals in HCMC, can provide reliable information on the prevalence of *H. pylori* antibiotic resistance and heteroresistance among Southern Vietnamese children.

5 | CONCLUSIONS

The alarming antibiotic resistance rates of *H. pylori*, especially to CLA among Vietnamese children are a serious issue, making them unsuitable for empiric treatment regimens. The high prevalence of multidrug resistance and the emergence of heteroresistant strains may pose major challenges in managing *H. pylori* infection in children. Therefore, obtaining multiple biopsies from both the gastric antrum and the corpus is crucial to improve the success of *H. pylori* culture and antibiotic susceptibility testing, which allows tailoring the treatment to individual contexts and monitoring of resistance evolution at the population level.

AUTHOR CONTRIBUTIONS

Tu Cam Nguyen participated in all research phases from ideation to manuscript writing. Phuong Ngoc Van Nguyen contributed to the study design, implementation, statistical analysis, and supervision. Giao Kim Ngoc Le and Bao Van Pham performed the culture. Dao Thi Hong Pham performed the PCR tests. Loan Thi Hong Nguyen and Thai Hoang Che contributed to data collection. Hiep Thanh Nguyen and Dinh Quang Truong contributed to the general supervisory research project management. Annie Robert and Patrick Bontems provided critical revision and shaped the manuscript. All authors approved the final version of the manuscript for publication.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to disclose.

DATA AVAILABILITY STATEMENT

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

ORCID

Tu Cam Nguyen https://orcid.org/0000-0001-8497-3739
Phuong Ngoc Van Nguyen https://orcid.org/0000-0002-0911-3242

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