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Helicobacter pylori Eradication Rate Using Stool Antigen Test in Vietnamese Children

A Prospective Multicenter Study

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ABSTRACT

Objectives: This study assessed the diagnostic value of a monoclonal immunoassay stool antigen test (HpSA) for *Helicobacter pylori (H. pylori)* infection and the eradication outcomes.

Methods: Children undergoing digestive endoscopy at 2 Children's Hospitals in Ho Chi Minh City were recruited. Treatment was offered to *H. pylori*-infected children. Stool samples were collected on the same day as the endoscopy procedure and after 6 weeks post-treatment for HpSA. Diagnostic value and optimal cutoff of HpSA were assessed using biopsy-based tests as the gold standard. Eradication was defined as a negative HpSA post-treatment. Ethical approval was obtained, and informed consent was signed by the participants.

Results: In total, 394 patients participated in the study. The most common symptoms were epigastric pain (74.6%) and vomiting (37.3%). *H. pylori* status was positive in 78% of patients (306/394), doubtful in 10.1%, and negative in 12.2%. HpSA was positive in 73.2% (142/194). Excluding doubtful infections, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of HpSA were 87.4%, 95.2%, 99.2%, 51.3%, and 88.4%, respectively. The optimal cutoff value of 0.148 provided similar accuracy to the recommended cutoff. The eradication rate was 56.1% in per-protocol analysis and 27.9% in intention-to-treat analysis. Treatment success was higher in boys, but lower among malnourished children and those infected with *cagA+* strains.

Conclusions: The HpSA is reliable for identifying *H. pylori* infection in epidemiological studies and assessing eradication outcomes. The low eradication rate highlights the need for an appropriate intervention strategy in Vietnamese children.

Key Words: *Helicobacter pylori*; monoclonal stool antigen test, biopsy-based tests, eradication treatment outcome, symptomatic children

What Is Known

- A monoclonal stool antigen test (HpSA) is recommended to confirm *Helicobacter pylori* eradication, but the performance of each test varies across populations.
- The treatment goal is 90% success on the first attempt.

What Is New

- The HpSA demonstrates high sensitivity (87%) and optimal specificity (100%) in Vietnamese children, independent of age, gender, nutritional status, and endoscopic lesions.
- A very low eradication rate of only 56.1% is observed in the per-protocol analysis and 27.9% in the intention-to-treat analysis.
- Boys have higher treatment success rates, while malnourished children and those infected with *cagA+* strains are associated with lower treatment success rates.

INTRODUCTION

Helicobacter pylori (*H. pylori*) causes gastroduodenal diseases and can develop into gastric cancer later in life (1). Vietnam has a high prevalence of *H. pylori* infection, which varies by geography

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The authors report no conflicts of interest.

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request. Some data have already been analyzed and published in a previous article (DOI: 10.1111/hel.13009).

The study was conducted according to the guidelines of the Declaration of Helsinki, approved by the Scientific Council of the Pham Ngoc Thach University of Medicine (No 2683/QĐ-TĐHYKPNT) and the local Ethics Committees of 2 hospitals (No 37/QĐ-BVNĐTP).

Written informed consent of parents/legal guardians was obtained before data and sample collection. Children older than 12 years also signed informed assent.

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(2,3). Timely and accurate diagnosis and effective eradication regimens are important to treat associated complications and prevent recurrence (4,5). Various invasive methods based on gastric biopsies (rapid urease test [RUT], histologic examination, culture, polymerase chain reaction [PCR]) and noninvasive methods (serology, urea breath test, and stool antigen tests) are available for diagnosing *H. pylori* infection (6).

The stool antigen test (HpSA) detects *H. pylori* through enzyme immunoassays with monoclonal or polyclonal antibodies (7). This method offers several advantages: it is easy to implement, cost-effective, reliable, and well-tolerated in young children (8,9), with high sensitivity (96.2%) and specificity (94.7%) (10). According to the latest European Society for Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) guidelines, a monoclonal HpSA is suitable to confirm *H. pylori* eradication in children and for epidemiological purposes (5).

However, each HpSA may perform differently due to variations in patient characteristics, the prevalence of *H. pylori* infection, and the type of strains. The diagnostic performance of each HpSA test should be evaluated in each population. Therefore, we undertook a study to assess the diagnostic value of a monoclonal HpSA for detecting *H. pylori* infection in our population. The secondary objective was to assess the success rate of eradication treatment in Vietnamese children.

MATERIALS AND METHODS

Study Population and Design

From October 2019 to May 2021, a prospective observational study was conducted on consecutive children aged 4 to 16 years undergoing upper gastrointestinal endoscopy because of alarm symptoms at 2 Children's Hospitals in Ho Chi Minh City (HCMC), Vietnam. These symptoms include persistent right upper quadrant pain, dysphagia, odynophagia, persistent vomiting, gastrointestinal blood loss, and involuntary weight loss, according to ESPGHAN guidelines (5). Children referred for interventional endoscopy, receiving proton-pump inhibitors (PPIs), histamine receptor blockers, antacids, bismuth salts within the previous 2 weeks, and antibiotics within the past 4 weeks before the endoscopy were excluded. Endoscopic lesions were described according to minimal standard terminology for digestive endoscopy (11). Demographic characteristics, *H. pylori* treatment history, nutritional status, and endoscopic findings were collected.

Written informed consent was obtained from parents/legal guardians of all participants, and informed assent was also signed by children over 12 years. The study was approved by the Scientific Council of the Pham Ngoc Thach University of Medicine (No2683/QĐ-TĐHYKPNT) and the local Ethics Committees of both hospitals (No37/QĐ-BVNĐTP).

H. pylori Diagnostic Biopsy-Based Tests

In each patient, gastric biopsies were taken for *H. pylori* culture, histological examination, RUT, PCR of *ureA* gene, cytotoxin-associated gene A (*cagA)* gene, and vacuolating cytotoxin A (*vacA)* genotypes.

For *H. pylori* culture, 1 antral and 1 corpus biopsy were separately processed and plated on special media following the standard procedure, then incubated in a microaerophilic environment at 35°C for up to 14 days. Thereafter, *H. pylori* identification was performed by positive urease, catalase, and oxidase tests.

Antimicrobial susceptibility testing (AST) was performed for amoxicillin (AMO), clarithromycin (CLA), metronidazole (MET), levofloxacin (LEV), and tetracycline (TET) using the *E* test (BioMérieux, Belgium). The resistant breakpoints for *H. pylori* were based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (Version 13.0), using the minimum inhibitory concentration value.

Two other gastric biopsies were fixed in 10% buffered formalin for histopathological examination. Histological findings were categorized using the updated Sydney classification system (12). A final antral biopsy was used for a RUT (UreaseNS, VietaCorporation, Vietnam) and *H. pylori* urease gene detection by Real-time PCR (AccuPidHPDetection Kit, KTBiotech, HCMC, Vietnam). Additionally, the *cagA* gene and *vacA* genotypes were identified using multiplex PCR (AccuLiteHPGenotyping Kit, KTBiotech, HCMC, Vietnam). The positive control used DNA from 2 *H. pylori* strains, strain J99 (ATCC 700824 *cagA+*, *s1m1*) and strain Tx30A (ATCC 51932, *cagA−*, *s2m2*), while the negative control did not contain *H. pylori* DNA.

H. pylori infection before treatment was diagnosed if a positive culture or a combination of histological evidence along with a positive RUT or a positive PCR of the *ureA* gene according to ESPGHAN/NASPGHAN criteria (5). *H. pylori* was considered doubtful if only 1 biopsy-based test was positive with a negative culture and negative if all 4 biopsy-based tests were negative.

Stool Antigen Immunoassay Test

Stool samples were collected at the time of endoscopy and at least 6 weeks after completing the eradication treatment. PPIs were stopped for at least 2 weeks and antibiotics for 4 weeks before stool collection. The samples were assessed for *H. pylori* using a monoclonal immunoassay stool test (Premier PlatinumHpSA PLUS, Meridian Bioscience, US). Stool samples were stored under 2°C to 8°C and sent to the laboratory within 24 hours. The specimens were centrifuged at 2750xG for 5 minutes and added to a test well. Positive control contained inactivated *H. pylori* and 10-mM phosphate-buffered solution with 0.02% Thimerosal. Negative control reagents contained a phosphate-buffered solution with Thimerosal. A peroxidase-conjugated monoclonal antibodies solution was added to all wells and incubated at room temperature for 1 hour. After washing, urea peroxide was then added to the antibody-coated wells. HpSA was then analyzed by single spectrophotometry (450 nm) within 15 minutes. As recommended by the manufacturer, an optical density (OD) ≥0.14 indicated positive HpSA results. The OD of the positive control was ≥0.64, and <0.14 for the negative control.

Treatment Regimens and Follow-up

Treatment was offered to patients with confirmed *H. pylori* infection. These eradication regimens were not standardized, but complied with national guidelines. When AST was available, a treatment tailored to susceptibility was prescribed. When AST was not available, the first-line eradication regimen was esomeprazole, AMO, MET, with/without bismuth salts (EAM/EAMB), or a rescue therapy in non-naïve patients, with a weight-based dosage according to ESPGHAN/NASPGHAN guidelines and national protocols. The duration of treatment was 14 days, with PPIs for 8 weeks in case of ulcers. A first follow-up visit was planned 2 weeks after prescribing the eradication regimen. Treatment compliance was assessed by interviews with patients and their parents. A second visit was scheduled at least 6 weeks after completing the treatment to reevaluate the *H. pylori* status using HpSA. *H. pylori* eradication was confirmed by a negative HpSA.

Statistical Analysis

The minimum sample size to assess the diagnostic value of the HpSA, calculated by the formula developed by Buderer (13), was 104. The calculation was based on an expected sensitivity of 96.6% and a specificity of 94.9% according to a previous Vietnamese study (14), a precision of 10%, a confidence interval (CI) of 95%, and a prior prevalence of *H. pylori* infection of 80%.

Sensitivity, specificity, PPV, NPV, and accuracy of HpSA were assessed using biopsy-based tests as a reference for *H. pylori* infection. The area under the receiver operating characteristics (ROC) curve was computed, and the cutoff maximizing Youden J was determined. Association between HpSA post-treatment and categorical variables (demographic characteristics, treatment history, nutritional status, and endoscopic findings) were assessed by χ^2 test or Fisher exact test and by Cochran–Armitage trend test for ordered categorical variables. All variables with the number of patients greater than 10 were included in the multiple logistic regression to identify potential factors associated with a negative HpSA post-treatment. All statistical tests were 2-tailed, and the significance level was set to 0.05. Stata SE 17.0 (Stata Corporation, TX) software was used for statistical analyses.

RESULTS

Patient Characteristics

Between October 2019 and May 2021, a total of 394 children were eligible and included. Endoscopy was indicated for 1 or more symptoms, including epigastric pain (74.6%; 294/394), persistent vomiting (37.3%), weight loss (31.5%), dyspepsia (14%), anemia (14.5%), and upper GI bleeding (9.6%). *H. pylori* infection was positive in 77.7% of patients (306/394), doubtful in 10.1% (40/394), and negative in 12.2% (48/394). A total of 280/306 *H. pylori*-positive patients received eradication therapy. Among them, 214 cases underwent the first follow-up visit. Subsequently, only 139 patients returned for a second visit. The study workflow is shown in Figure 1. The baseline characteristics and endoscopic findings of all study patients (n = 394), *H. pylori*-positive children (n = 306), those with pretreatment HpSA (n = 194), and those with post-treatment HpSA results (n = 139) are summarized in Table 1.

Diagnostic Value of HpSA

Among the 394 enrolled children, 194 had available stool specimens on the same day of endoscopy. These children's baseline characteristics were similar to the entire study participant's (Table 1). Among them, 151 were *H. pylori* positive, 22 had doubtful status, and 21 were negative.

Following the manufacturer's cutoff value, the overall HpSA positivity was 73.2% (142/194). HpSA was positive in 87.4% (132/151) of *H. pylori*-positive children, 40.9% (9/22) of doubtful, and 4.8% (1/21) of negatives. The median OD of HpSA was 2.22 (interquartile range (IQR), 0.68–3.72) in *H. pylori*-positive children, 0.05 (IQR, 0.04–2.54) in doubtful infections, and 0.04 (IQR, 0.03–0.04) in *H. pylori*-negative children.

Excluding the doubtful infections, the sensitivity, specificity, PPV, NPV, accuracy, and area under curves of the HpSA were: 87.4% (95% CI, 81.0–92.3), 95.2% (95% CI, 76.2–99.9), 99.2% (95% CI, 95.9–100), 51.3% (95% CI, 34.8–67.6), 88.4% (95% CI, 82.6–92.8), and 0.913 (95% CI, 0.860–0.967), respectively. The positive likelihood ratio (LR+) was 18.4 (95% CI, 2.71–124.4), and the negative likelihood ratio (LR−) was 0.13 (95% CI, 0.09–0.20). Based on the ROC curve, the optimal cutoff value was 0.148, with a sensitivity of 87% and a specificity of 100%. No significant differences in the area under curves of HpSA were found among age groups, gender, nutritional status, and endoscopic findings.

Antibiotic Resistance

AST was available for 116 out of 280 treated patients (41.4%). Of these, 93 (80.2%) were infected by strains resistant to at least 1 antibiotic. Specifically, the resistance rate of CLA, MET, LEV, AMO, and TET was 72.4% (84/116), 39.7%, 60.3%, 25.9%, and 1.7%, respectively. Double resistance accounted for 25.9 % (30/116), with the highest prevalence observed in CLA+LEV (19.0%, 22/116), followed by CLA+MET (5.2%, 6/116) and AMO+CLA (1.7%, 2/116). Triple resistance was found in 27.6% (32/116) of patients, with CLA+MET+LEV being the most common pattern (15.5%, 18/116). Quadruple resistance was observed in 12.9% (15/116) of patients, mostly with AMO+CLA+MET+LEV (12.0%, 14/116). No significant difference in antibiotic resistance was observed between age groups, gender, living area, nutritional status, endoscopic findings, *cagA*, and *vacA* genotypes.

Heteroresistance was observed in 6% of the patients (7/116). Of these, 2 patients had heteroresistance to AMO, 2 to MET, 2 to LEV, and 1 patient had heteroresistance to both CLA and LEV.

Therapeutic Regimens

A treatment was prescribed in 280 patients of which peptic ulcer disease (PUD) was identified in 65 (23.2%), erosion in 28 (10%), nodularity in 176 (62.9%), and erythema in 11 (3.9%). The most used first-line regimens were esomeprazole, AMO, and MET (EAM) (65.4%, 183/280), followed by esomeprazole, AMO, MET, and bismuth subcitrate (EAMB) (25.7%), esomeprazole, AMO, CLA (EAC) (4.6%), and other regimens (LEV-based or TET-based regimens) (4.3%) (Fig. 1).

Out of the total 280 patients treated for *H. pylori*, 62 patients were previously treated. The indications for retreatment were recurrent PUD (15; 24.2%), erosion (8; 12.9%), nodularity (38; 61.3%), and erythematous gastritis (1; 1.6%). Among them, AST results were obtained in 27 patients whose treatment regimens were EAM (11;40.7%), EAMB (7; 25.9%), EAC (4; 14.8%), TET-based regimens (3; 11.1%), and LEV-based regimens (2; 7.4%). In the absence of AST, the following empiric regimens were used: EAM (20; 57.1%), EAMB (14; 40.0%), and LEV-based regimens (1; 2.9%).

Outcome of Eradication Treatment

Among 139 patients with HpSA results post-treatment, the mean age was 9.7±2.6 years, and 50.4% were boys. The proportion of boys to girls increased with age (P = 0.007), with 43.3% in the group under 11 years old and 66.7% in the group older than 11 years. Peptic ulcers accounted for 25.2% (35/139) and were observed more frequently in boys (68.6%; 24/35) than in girls (31.4%) (P = 0.01). Other endoscopic lesions were detected, such as erosions (17; 12.2%), nodularity (84; 60.4%), and erythema (3; 2.2%). The nonnaïve patients accounted for 23.7% (32/135). The treatment regimens were EAM (69.8%), EAMB (21.6%), EAC (3.6%), TET-based regimens (3.6%), and LEV-based regimens (1.4%).

The overall eradication rate was 56.1% (78/139) in the per-protocol analysis and 27.9% (78/280) in the intention-to-treat analysis. The per-protocol eradication rate with EAMB was higher than with EAM or EAC, but the differences were not statistically significant (Fig. 2).

The per-protocol eradication rate for the EAM and EAMB regimen was only 56.3% (18/32) when *H. pylori* was susceptible to both AMO and MET. Detailed eradication rates for each regimen, categorized by antibiotic susceptibility through per-protocol and intention-to-treat analysis, are presented in Table 2.

Concerning adverse effects, 14.5% (31/214) experienced side effects, including fatigue (7.0%), diarrhea (3.8%), nausea and vomiting (3.8%), and other symptoms (loss of appetite, headache, dizziness) (2.4%). Patients may have experienced more than 1 adverse event. Regarding treatment compliance, 18.2% (39/214) of patients reported missing at least 1 dose during treatment.

Eradication success was higher in boys, but lower in underweight children and *cagA*-positive *H. pylori* patients. Among the nonnaïve patients, the eradication rate was 59.4% compared to 55.3% among naïve patients, and this difference did not reach statistical

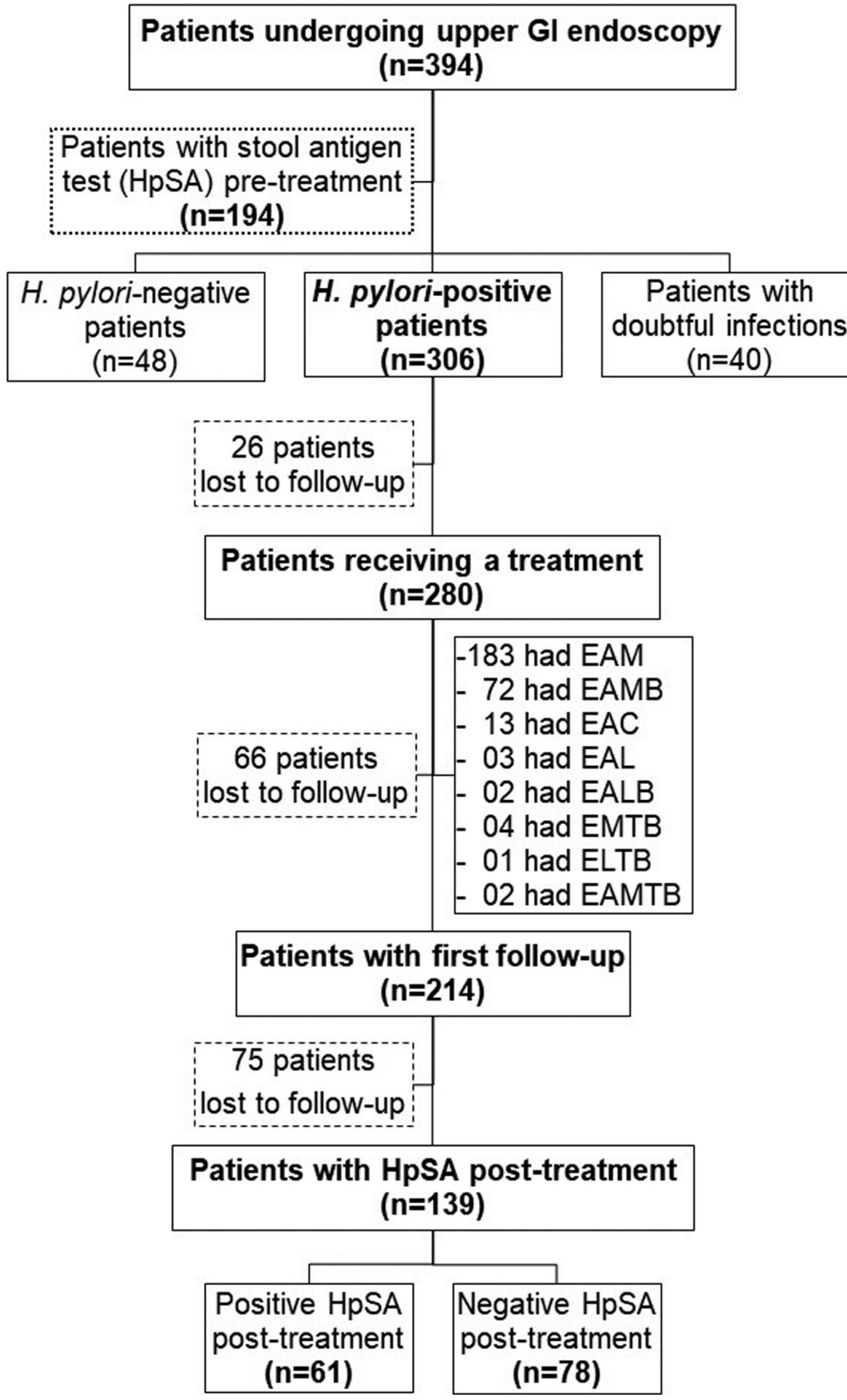

FIGURE 1. Study flow chart. A, amoxicillin; B, bismuth subcitrate; C, clarithromycin; E, esomeprazole; GI, gastrointestinal; HpSA, *H. pylori* stool antigen test; L, levofloxacin; M, metronidazole; T, tetracycline.

TABLE 1. Characteristics of study population

Characteristics	All patients N = 394	*H. pylori*-positive children N = 306	Patients with HpSA before treatment N = 194	Patients with HpSA after treatment N = 139
Age (mean age ± SD)	9.4±2.5	9.5±2.5	9.6±2.7	9.7±2.6
Age group				
<6 years	25 (6.4)	19 (6.2)	15 (7.7)	7 (5.0)
6–<11 years	267 (67.8)	205 (67.0)	120 (61.9)	90 (64.8)
>=11 years	102 (25.9)	82 (26.8)	59 (30.4)	42 (30.2)
Gender				
Boys	177 (44.9)	163 (53.3)	92 (47.4)	70 (50.4)
Girls	217 (55.1)	143 (46.7)	102 (52.6)	69 (49.6)
Living area				
Provinces	232 (58.9)	163 (53.3)	109 (56.2)	58 (41.7)
HCMC	162 (41.1)	143 (46.7)	85 (43.8)	81 (58.3)
+ Rural	102 (63.0)	89 (62.2)	61 (71.8)	49 (60.5)
+ Urban	60 (37.0)	54 (37.8)	24 (28.2)	32 (39.5)
History of *H. pylori* treatment				
Yes	101 (26.3)	69 (23.2)	53 (27.9)	32 (23.7)
No	283 (73.7)	228 (76.8)	137 (72.1)	103 (76.3)
Nutritional status				
Malnutrition	76 (19.3)	54 (17.6)	31 (16.0)	22 (15.8)
Normal weight	208 (52.8)	159 (52.0)	109 (56.2)	72 (51.8)
Obesity/overweight	110 (27.9)	93 (30.4)	54 (27.8)	45 (32.4)
Endoscopic findings				
Ulcers	77 (19.5)	68 (22.2)	37 (19.1)	35 (25.2)
Erosions	35 (8.9)	29 (9.5)	30 (15.5)	17 (12.2)
Nodularity	234 (59.4)	192 (62.8)	91 (46.9)	84 (60.4)
Erythema	48 (12.2)	17 (5.6)	36 (18.6)	3 (2.2)

HCMC = Ho Chi Minh City; HpSA = Helicobacter pylori stool antigen test.

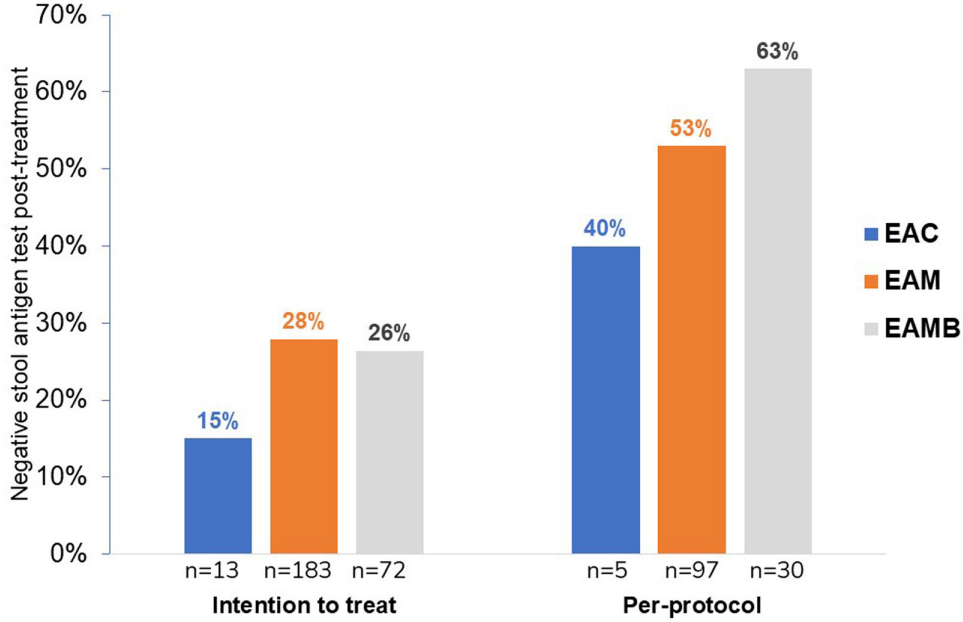

FIGURE 2. *Helicobacter pylori (H. pylori)* eradication rate with EAC, EAM, EAMB, by intention to treat, and per-protocol analysis. A, amoxicillin; B, bismuth subcitrate; C, clarithromycin; E, esomeprazole; M, metronidazole.

TABLE 2. Eradication rate according to antibiotic susceptibility

	Intention-to-treat		Per-protocol	
	N*	Success (%)	N*	Success (%)
EAM	64	23 (35.9)	38	23 (60.5)
Both susceptible	41	16 (39.0)	28	16 (57.1)
Resistance to A	7	3 (42.9)	3	3 (100)
Resistance to M	11	2 (18.2)	4	2 (50.0)
Both resistance	5	2 (50.0)	3	2 (66.7)
EAMB	28	4 (14.3)	9	4 (44.4)
Both susceptible	9	2 (22.2)	4	2 (50.0)
Resistance to A	4	1 (25.0)	1	1 (100)
Resistance to M	7	1 (14.3)	3	1 (33.3)
Both resistance	8	0 (0.0)	1	0 (0.0)
EAC	13	2 (15.4)	5	2 (40.0)
Susceptible	10	2 (20.0)	4	2 (50.0)
Resistance to A	1	0 (0.0)	0	0 (0.0)
Resistance to C	2	0 (0.0)	1	0 (0.0)
Both resistance	0	0 (0.0)	0	0 (0.0)

EAC = esomeprazole, amoxicillin, clarithromycin; EAM = esomeprazole, amoxicillin, metronidazole; EAMB = esomeprazole, amoxicillin, metronidazole, bismuth subcitrate.
*Total number of treated patients.

significance. In addition, no association between eradication rate was found between living areas, endoscopic findings, *vacA* genotypes, antibiotic susceptibility, adverse effects, and treatment compliance (Table 3).

DISCUSSION

Routine testing to confirm *H. pylori* eradication is necessary for patients who receive treatment to prevent recurrence (5,15). Firstly, we evaluated the validity of a monoclonal HpSA for diagnosing *H. pylori* using biopsy-based tests as a reference method. Our findings demonstrated high sensitivity (87.4%) and excellent specificity (95.2%) when using the cutoff value suggested by the manufacturer. Using the optimal cutoff determined by the ROC curve, the sensitivity is similar, but the specificity reaches 100%. Using the same HpSA test, a study conducted with 232 children in Northern Vietnam in 2008 reported a sensitivity of 96.6% compared to positive *H. pylori* culture and a specificity of 94.9% compared to negative serology (14). Another study in Spanish adults indicated a lower sensitivity of 72% and a similar specificity of 95% (16). However, using histological examination as the reference standard, a study conducted in Indonesia showed a sensitivity of 91.7% but a low specificity of 22.2% (17) while a study in Iraq found a low sensitivity of 36% but a specificity of 100% (18). Some meta-analyses showed a pooled sensitivity of 83% to 96%, and a specificity of 95% to 97% (9,10,19). The differences in the diagnostic performance of HpSA across populations are due to variations in test characteristics, cutoff values, reference gold standards, prevalence of *H. pylori* infection, and medication usage (10). This highlights the importance of validating the HpSA within each specific population.

Additionally, finding the optimal cutoff value for HpSA in a distinct population is also important. From the ROC curve, the optimal cutoff value was 0.148, giving comparable accuracy to the manufacturer's cutoff point of 0.14. Furthermore, no difference in HpSA performance between age group, gender, nutritional status, and endoscopic findings was observed. These results indicate the consistent diagnostic value of HpSA across multiple Vietnamese pediatric subpopulations.

However, HpSA had a false-negative rate of 12.6% and a false-positive rate of 4.8%. This is possibly due to low *H. pylori* antigen concentrations in stools (20). Stool sample quality and storage conditions also affect HpSA results (21). However, with an LR+ of 18.4 and an LR− of 0.13, our results indicated that this HpSA provided convincing diagnostic evidence. Also, with a high PPV (99.2%), it is useful to confirm *H. pylori* infection in community-based research. Therefore, this monoclonal HpSA holds relevant diagnostic value in clinical settings and epidemiological studies in Vietnamese children.

Our secondary objective was to assess the success rate of eradication regimens. Our data showed remarkably low eradication rates, with only 56.1% in the per-protocol analysis and 27.9% in the intention-to-treat analysis. These rates fall significantly far from the desired goal of achieving a cure rate exceeding 90% on the first attempt (5). Success rates are similar in naïve and non-naïve patients.

The main factor contributing to low success rates can be antibiotic resistance. A recent study on Vietnamese children showed that *H. pylori* had an alarming level of antibiotic resistance, with the emergence of multiresistant and hetero-resistant strains (22). In this study, among 116 patients with AST results, 80.2% carried *H. pylori* strains resistant to at least 1 antibiotic. CLA had the highest resistance rate (72.4%), followed by LEV (60.3%), MET (39.7%), and AMO (25.9%). International guidelines recommend avoiding empiric CLA-based regimens if regional resistance surpasses 15% (4,5,23). The World Health Organization has classified CLA-resistant *H. pylori* infection as a high threat, emphasizing the need for antibiotic research development and good stewardship (24). In children, AMO, CLA, and MET are the most used antibiotics in *H. pylori* treatment while the use of LEV and TET are limited due to their negative impact on children's bone growth. Our data also found the emergence of heteroresistance (6%), indicating mixed infection that could also contribute to treatment failure (25). The coexistence of both susceptible and resistant strains in the same patient can make it difficult to effectively eradicate if only the susceptible strains were detected on gastric biopsies.

TABLE 3. Eradication rate accessed by a negative stool test according to characteristics of 139 children treated for *Helicobacter pylori* (*H. pylori*) infection

Characteristics	N	Success %	OR	*P*	aOR	*P*
Age (years)				0.09		0.09
<11	97	60.8	1		1	
≥11	42	45.2	0.53 (0.26–1.11)		0.41 (0.15–1.14)	
Gender				0.02		0.02
Boys	70	65.7	2.22 (1.12–4.39)		3.04 (1.20–7.73)	
Girls	69	46.4	1		1	
Living area				0.59		0.71
Provinces	58	53.5	1		1	
Ho Chi Minh City	81	58.0	1.20 (0.61–2.37)		1.18 (0.49–2.83)	
+ Rural	49	53.1	1			
+ Urban	32	65.6	1.69 (0.67–4.24)	0.26		
History of pretreatment*				0.69		0.22
Yes	32	59.4	1.18 (0.53–2.64)		2.00 (0.67–5.92)	
No	103	55.3	1		1	
Malnutrition				0.01		0.03
Yes	22	31.8	0.30 (0.11–0.80)		0.27 (0.08–0.87)	
No	117	60.7	1		1	
Endoscopic findings				0.52		0.74
Ulcers	35	51.4	0.78 (0.36–1.67)		1.19 (0.44–3.23)	
Gastritis	104	57.7	1		1	
cagA status†				0.01		0.02
Positive	101	49.5	0.36 (0.16–0.83)		0.25 (0.08–0.78)	
Negative	37	73.0	1		1	
vacA genotypes†				0.73		0.69
s1/m1	44	54.6	0.60 (0.10–3.62)		0.70 (0.09–5.51)	
s1/m2	56	58.9	0.72 (0.12–4.25)		0.35 (0.04–2.94)	
s2/m2	1	100	1		1	
Mixed infections	31	48.4	0.47 (0.07–2.94)		0.46 (0.06–3.62)	
Incomplete *vacA*	6	66.7				
Antibiotic susceptibility to regimens‡				0.61		
Susceptible to 2 ATB	38	55.3	1			
Resistance to 1 ATB	16	68.8	1.78 (0.52–6.13)			
Resistance to 2 ATB	4	50.0	0.81 (0.10–6.36)			
Side effects§				0.66		0.99
Yes	19	63.2	1.26 (0.45–3.46)		0.99 (0.32–3.10)	
No	97	57.7	1		1	
Compliance with treatment‖				0.89		0.65
Yes	96	58.3	1.07 (0.40–2.86)		1.32 (0.40–4.29)	
No	20	60.0	1		1	

aOR = adjusted odds ratio; ATB = antibiotics; OR, odds ratio.
*Four missing data.
†One missing data.
‡Antimicrobial susceptibility testing results were available in 58 patients.
§Information on side effects was available on 115 patients.
‖Information on compliance with treatment was available on 116 patients.

Regarding eradication regimens, EAM and EAMB were prescribed in most patients (91.1%). A 14-day combination therapy including PPIs and 2 antibiotics (AMO and MET) was recommended (5,26–28). CLA was considered only if susceptibility was confirmed. LEV and TET were prescribed limitedly in older children and for cases with previous treatment failure. Second-line therapy was often determined by considering the initial regimen and antibiotic susceptibility if available (28). However, as shown in Table 3, among

patients with AST results, despite *H. pylori's* susceptibility to both antibiotics used in the treatment regimen, the per-protocol eradication rates were only 50% for EAC and EAMB, and 57.1% for EAM, respectively. This strongly suggests that noncompliance with therapy played also an important role in poor outcomes. Despite fairly good treatment compliance being observed in this study (81.8%), this information was unverifiable as recalled by the patient's parents. Similarly, the per-protocol eradication rate was nearly double the intention-to-treat rate. The considerable number of dropouts during follow-up visits may indirectly indicate poor compliance. This reemphasizes that treatment compliance is critical to the eradication outcomes. Although the loss of follow-up was due to the COVID-19 pandemic, the importance of compliance still cannot be dismissed. In a cohort of children, treatment compliance above 90% was strongly associated with successful outcomes (29). Enhancing medication compliance improved the eradication (30).

Furthermore, another factor that should also be considered is the necessity to treat patients without ulcers to avoid overtreatment that can lead to the development of antibiotic resistance. Eradication treatment is essential for patients diagnosed with *H. pylori*-positive PUD or erosions. It can be considered for patients with other gastritis (5,15). In this study, all patients were indicated for upper GI endoscopy due to the presence of warning signs indicating organic causes, with epigastric pain being the most common symptom (74.6%). Through endoscopy, PUD and erosions were detected in 33.2% of patients. For the remaining 66.8% of patients with nonulcer lesions, the guidelines propose engaging in a discussion with parents and children about the risks and benefits of treatment to facilitate decision-making (5). Nevertheless, parents opted to treat their children when the patient had persistent symptoms with the presence of gastritis signs. For clinicians, treatment was expected to prevent progression towards ulcer and gastric cancer in an area with high incidences of gastric cancer such as Vietnam (age-standardized rate >11.1 per 100 000 inhabitants) (31). As a result, most patients with *H. pylori* infection were treated, except those who lost to follow-up. Therefore, prescribing treatment when necessary, tailoring treatment, and improving compliance through patient education, counseling, reminders, and support will likely be critical to achieving higher eradication rates and reducing antibiotic resistance.

Interestingly, EAMB was more efficient than EAM and EAC, despite the high MET resistance rate (39.7%). Bismuth-based quadruple therapy showed effective eradication in clinical trials, even in patients with resistance to MET, or both CLA and MET (32–34). This could be related to the discrepancy between in vitro AST results and in vivo settings. Especially, clinical efficacy can be influenced by the synergistic effects of concomitant medications, especially between MET and bismuth. As a result, the EAMB regimen may be a viable option for empiric treatment in Vietnamese children, providing that optimal dosage, duration, and adherence to the treatment are ensured.

Other factors associated with eradication success were identified. Boys had a higher eradication rate, consistent with previous results (35,36). This could be explained by their better compliance with treatment as the incidence of PUD was significantly higher or their differences in immune response. Conversely, treatment success was lower among underweight children, possibly due to weakened immune systems and altered drug metabolism. Additionally, *cagA+* strains had a significantly lower eradication rate, contrary to other studies (37,38). Various genotypes of *H. pylori* may lead to different clinical outcomes in distinct populations.

Our main limitation was a high loss to follow-up due to COVID-19 disruptions since February 2020. Second, data on adverse events and treatment compliance relied on subjective information since there was no diary or drug accountability. Third, the treatment regimens were not standardized but chosen by physicians following local protocols. Also, HpSA was the only method used to assess the eradication, despite its low NPV (51.3%), which may overestimate the eradication rates. However, our efforts were made to optimize HpSA accuracy by excluding patients who received antibiotics and PPIs, using single spectrophotometry instead of visual assessment, and using 2 diagnostic biopsy-based methods (if the culture was negative) as a reference. Therefore, this multicenter study reflects the diagnostic performance of HpSA and provides practical insights into treatment regimens chosen by physicians and the actual eradication rates in HCMC.

CONCLUSIONS

The monoclonal HpSA is highly sensitive and specific, making it valuable in detecting *H. pylori* for epidemiological purposes and for predicting post-treatment eradication status, although it may slightly overestimate the success of the outcome. Furthermore, our results demonstrate poor treatment outcomes and high antibiotic resistance, highlighting the need for effective intervention strategies to improve the treatment effectiveness in Vietnamese children.

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