

# *Helicobacter pylori* Antimicrobial Susceptibility Testing-Guided Eradication Therapy in the Southeast Region of China: A Retrospective Study

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**Background and Aim:** Antibiotic resistance of *Helicobacter pylori* is increasing worldwide, lowering its efficacy in eradication therapy and posing a serious threat to human health. This study evaluated *H. pylori* resistance to antibiotics in the southeast region of China and explored factors related to eradication failure guided by antimicrobial susceptibility testing (AST).

**Methods:** In this retrospective study, patients who tested positive underwent gastroscopy, and *H. pylori* infection was confirmed by histological staining and *H. pylori* culture. We determined the rate of *H. pylori* antibiotic resistance, success rate of AST-guided eradication therapy, and risk factors associated with treatment failure.

**Results:** Among the 210 enrolled patients, 188 (89.5%) had successful cultures, and 183 (87.1%) underwent AST. The most common antibiotic resistance was to metronidazole and clarithromycin (89.6%), followed by levofloxacin (68.3%), and amoxicillin (14.2%). Furazolidone (3.0%) and tetracycline (0.5%) showed relatively low resistance rates. There were no statistically significant differences in the rates of resistance to MET, LEV, or AMX between naive and non-naive patients. However, CLA resistance rates in non-naive patients were significantly higher than those in naive patients. The overall success rate of AST-guided therapy was high and showed no significant difference between first-line and rescue therapy. Sex, age, prior therapy, and proton pump inhibitors (PPIs) or potassium-competitive acid blockers (P-CABs) use were not significantly associated with an increased risk of eradication failure in AST-guided therapy.

**Keywords:** Antibiotic resistance, Culture, *Helicobacter pylori*, Eradication therapy.

## Introduction

*Helicobacter pylori* (*H. pylori*) is a globally prevalent pathogen that colonizes the stomach. Persistent infection may lead to various gastrointestinal diseases including chronic gastritis, peptic ulcer, gastric cancer, and MALT lymphoma.<sup>1,2</sup> Its prevalence is significantly higher in developing countries than in developed countries.<sup>3</sup> Therefore, the eradication of *H. pylori* is a promising method to prevent gastric cancer in a cost-effective manner.

Unfortunately, the eradication rate has been decreasing owing to the emergence of antibiotic resistance strains.<sup>4</sup> Antibiotic resistance has reached alarming levels worldwide, especially nitroimidazole, clarithromycin and levofloxacin resistance. The pooled prevalence of primary resistance ranged from 21% to 37% for clarithromycin, 51% to 83% for metronidazole, and 18% to 42% for levofloxacin in recent 10 years.<sup>5</sup> It is characterized by the involvement of diverse mechanisms, including mutagenic modifications in drug target sites, membrane permeability variations, biofilm formation, and an abundance of efflux pump systems.<sup>6</sup> Thus, the prevalence of antibiotic resistance makes it more difficult for traditional empirical therapy to successfully eradicate *H. pylori*. Recent reports show that

antimicrobial susceptibility testing (AST)-guided treatment improves the bacterial eradication rates.<sup>7,8</sup> *H. pylori* AST is performed by culturing bacteria from gastric biopsies during endoscopic examination and determining the minimum inhibitory concentration of antibiotics required to inhibit bacterial growth.<sup>9</sup> Current guidelines recommend that patients who fail more than one course of *H. pylori* treatment undergo culture and AST to select susceptible antibiotics for tailored therapy.<sup>10</sup> Hence, drug susceptibility testing of *H. pylori* is crucial for successful eradication therapy.

Recent studies have shown a relatively lower success rate (around 90%) in *H. pylori* cultures compared to UBT, which limited its application.<sup>11</sup> In addition, the eradication rate based on AST and its possible impact factors have not been studied extensively. This study aimed to investigate the antibiotic resistance of *H. pylori* in southeastern China, explore factors related to therapy failure based on AST, and improve the eradication rate of *H. pylori*.

## Materials and Methods

### Patients

This study included patients diagnosed with *H. pylori* infection at The First Affiliated Hospital of Zhejiang University School of Medicine (Hangzhou, China) between November 2021 and December 2023. Electronic medical records, laboratory test results, endoscopy results, and histological results were also obtained. All the patients who underwent endoscopy with *H. pylori* culture and AST were retrospectively identified. Eradication therapy was performed by physicians based on *H. pylori* culture and AST results, medication allergy history, and previous antibiotic exposure.

The inclusion criteria were as follows: (1) male and female patients aged 18–70 years; (2) active *H. pylori* infection was detected using the urea breath test (<sup>13</sup>C-UBT or <sup>14</sup>C-UBT); (3) no use of antibiotics, bismuth, H2 receptor antagonists, or PPIs in the past 4 weeks; (4) agreeing to perform gastric mucosal biopsy for *H. pylori* culture and AST, and signing the informed consent form; and (5) successful *H. pylori* eradication was confirmed by urea breath test results >4 weeks after completion of treatment. The exclusion criteria were as follows: (1) gastrointestinal emergencies such as gastric perforation and peptic ulcer bleeding or (2) having taken bismuth, PPIs, H2 receptor antagonists, and antibacterial drugs in the previous month.

Prior to the gastroscopy examination, all participants were informed that their data might be used potentially for future research, and subject information would be anonymized. The study was carried out in accordance with the Declaration of Helsinki and approved by the Clinical Research Ethics Committee of the First Affiliated Hospital, the Zhejiang University School of Medicine (IIT20230843A). Written consent was not required because of the retrospective observational design of the study.

### *H. pylori* Culture and Antimicrobial Susceptibility Testing

Gastric mucosal tissues were obtained by experienced endoscopists during routine gastroscopy, inoculated into Columbia blood agar (Zhejiang Chingoo Biotech Co., Ltd), and cultured in a microaerobic environment (5% O<sub>2</sub>, 10% CO<sub>2</sub>, 85% N<sub>2</sub>, ANOXOMAT MARK II system) at 37 °C for 3–5 days in average (incubation time of non-*H. pylori*-growth samples were extended up to 7 days). *H. pylori* strains were confirmed by positive oxidase, catalase, and urease tests. Further confirmation of positive isolates was detected by antigen tests with the H. PYLORI ANTIGEN RAPID TEST produced by Abbott.

AST of *H. pylori* was performed using six antibiotics, which were tested by E-test strips (BIO-KONT, Wenzhou, China) according to the Etest<sup>®</sup> Application Guide (16273B - en - 2012/07) of bioMérieux SA (supplementary\_inserts\_-\_16273\_-\_b\_-\_en\_-\_eag\_-\_etest\_application\_guide-3.pdf (biomerieux-USA.com)). The *H. pylori* standard strain NCTC11637 was used as the quality control strain of E-test strips. The minimum inhibitory concentration (MIC) breakpoints for resistance are >0.25 mg/L for clarithromycin (CLA), >1 mg/L for levofloxacin (LEV), >0.125 mg/L for amoxicillin (AMX), >8 mg/L for metronidazole (MET), and >1 mg/L for tetracycline (TET), based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST Version 13.0) guidelines. The resistance cutoff MIC for Furazolidone (FR) was defined as >2 mg/L.<sup>12</sup>

## Statistical Analysis

Statistical analyses were performed using the SPSS, version 22.0. Categorical variables are displayed as frequencies and proportions (%). Continuous variables are expressed as means and standard deviations (SD). Continuous variables were compared using Student's *t*-test or one-way ANOVA. Categorical variables were compared using chi-square test. Stepwise logistic regression analysis was performed to examine the relationship between *H. pylori* eradication failure and the risk factors. Statistical significance was set at  $P < 0.05$ .

## Results

### Patient Characteristics

A total of 222 outpatients with a possible positive *H. pylori* infection by UBT were enrolled in this study. The patients underwent gastroscopy, and the two congested antrum and corpus biopsies were obtained for *H. pylori* culture. Twelve patients were excluded because of false-positive UBT results according to endoscopic and pathological findings. Of the 12 patients with false-positive infections, eight were confirmed to have autoimmune gastritis (AIG). Peptic ulcers were observed in 16.2% (34/210) of the 210 patients. Pre-neoplastic lesions, including intestinal metaplasia or dysplasia, were observed in 32.9% (69/210) of patients. Of the 210 patients, 185 received at least one course of *H. pylori* eradication therapy. Other clinical characteristics are shown in [Table 1](#).

### *H. pylori* Culture

Among 210 infected patients, 188 had positive culture results, yielding a sensitivity of 89.5%. The rate of UBT positivity was 94.6%. These results showed that the positivity rate of *H. pylori* culture was not inferior to that of the UBT ( $P = 0.05$ , [Table 2](#)). Finally, antibiotic susceptibility test results were obtained for 183 patients and included in further analyses ([Figure 1](#)).

### Primary and Secondary *H. pylori* Antibiotic Resistance Rates

The overall rates of resistance to CLA, MET, LEV, AMX, FR, and TET were 89.6%, 89.6%, 68.3%, 14.2%, 3.0%, and 0.5%, respectively. Among the 25 naive patients, the primary resistance rate was the highest for MET (92.0%, 23/25), followed by LEV (64.0%, 16/25), CLA (56.0%, 14/25), and AMX (4.0%, 1/25). There was no primary resistance to FR and TET. Among the 158 non-naive patients, the secondary resistance was higher in CLA (94.9%, 150/158), then MET

**Table 1** Baseline Characteristics of Study Cohort with Helicobacter Pylori Infection

Characteristics	Cases (n = 210)	Percentage
<b>Gender</b>		
Male	88	41.9%
Female	122	58.1%
<b>Age</b>		
<40	82	39.0%
40–60	102	48.6%
>60	26	12.4%
<b>Endoscopic and pathological finding</b>		
Peptic ulcer	34	16.2%
Pre-neoplastic lesions	69	32.9%
Neoplastic lesions	0	0
<b>Eradication attempts</b>		
Primary	25	11.9%
Rescue	185	88.1%

**Table 2** Analysis of *H. Pylori* Diagnostic Results of UBT and *H. Pylori* Culture

Method	Positive	Negative	P
UBT	210	12	0.05
<i>H. pylori</i> culture	188	22	

**Abbreviation:** UBT, urea breath test.

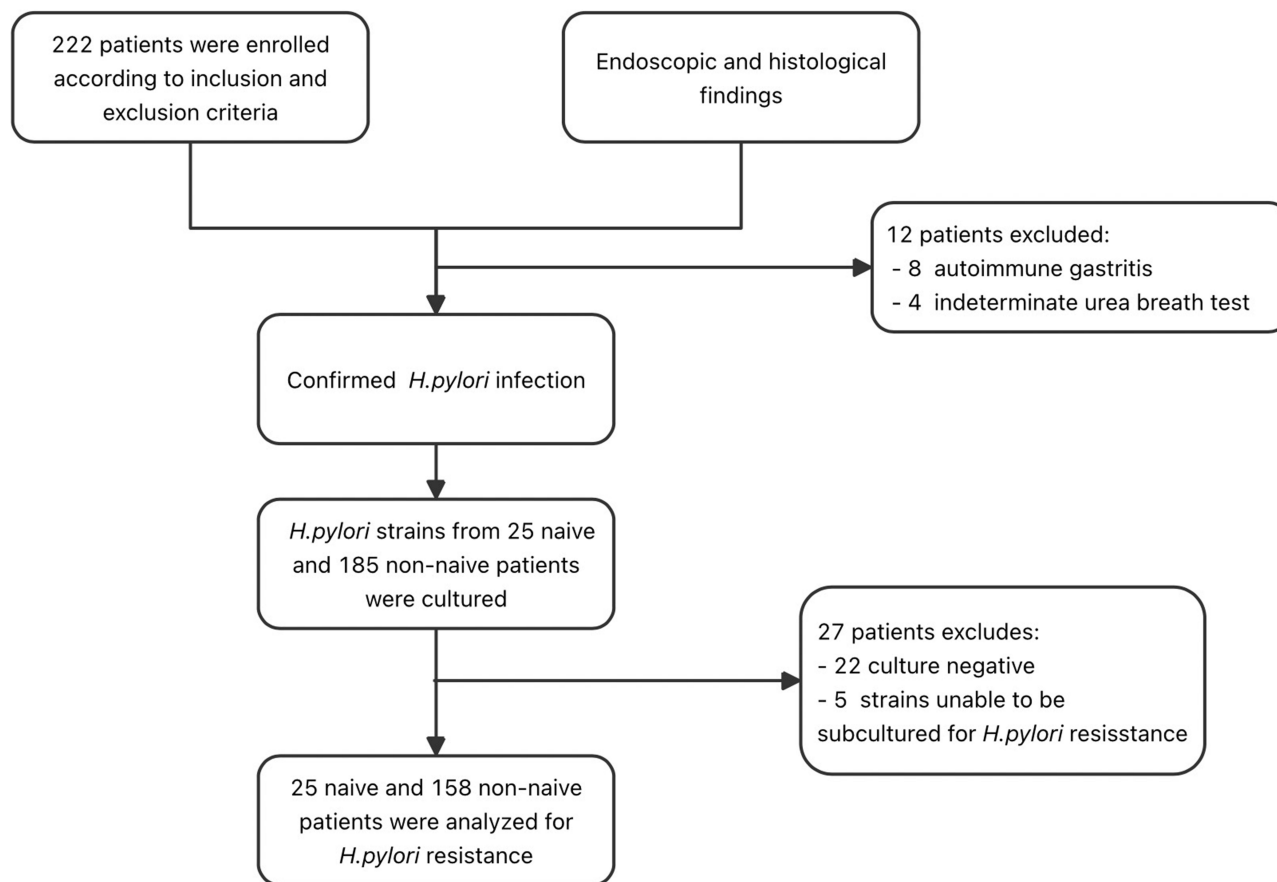
(89.2%, 141/158), LEV (69.0%, 109/158), AMX (15.8%, 25/158), FR (3.1%, 3/98), and TET (0.6%, 1/158). There were no statistically significant differences in the rates of resistance to MET, LEV, or AMX between the naive and non-naive patients. However, CLA resistance rates in non-naive patients were significantly higher than those in naive patients (94.9% vs 56.0%,  $P < 0.001$ , Table 3). Gender, age, and endoscopy findings were similar between the naive and non-naive patients (Supplementary Table 1).

## *H. pylori* Eradication Rates

Following *H. pylori* culture and AST, eradication was successful in 176 (96.2%) of the 183 patients. For the naive patients, the eradication rate was 96.0% (24/25). Furthermore, 96.2% (152/158) of the non-naive patients had successful eradication of *H. pylori*. Antibiotic combination according to AST results were preferred elaborately (Supplementary Table 2).

## Factors Associated with Eradication Rates

We further performed a univariate logistic regression analysis on 183 successfully eradicated cases and seven patients with failure to identify potential associated factors. As summarized in Table 4, the analysis confirmed that sex, age, prior



**Figure 1** Study flowchart.

**Table 3** Antibiotic Resistance Results of *H. pylori* Isolates

Antibiotic	All Patients	Naive patients		Non-Naive Patients		P
		Sensitive	Resistant	Sensitive	Resistant	
CLA	183	11	14	8	150	<0.001
MET	183	2	23	17	141	0.674
LEV	183	9	16	49	109	0.618
AMX	183	24	1	133	25	0.116
FR	109	11	0	95	3	0.556
TET	183	25	0	157	1	0.690

**Notes:** naive patients: patients who did not receive *H. pylori* eradication; non-naive patients: patients who received *H. pylori* eradication more than once.

**Abbreviations:** CLA, clarithromycin; MET, metronidazole; LEV, levofloxacin; AMX, amoxicillin; FR, Furazolidone; TET, tetracycline.

**Table 4** Risk Factors of Eradication Success

Factors	Eradication Rate	Univariate Analysis OR (95% CI)	P value
<b>Gender</b>			
Male	72/76 (94.7%)	1 (Reference)	0.400
Female	104/107 (97.2%)	1.926 (0.418–8.866)	
<b>Age</b>			
<40	66/69 (95.7%)	1 (Reference)	0.934
40–60	88/91 (96.7%)	1.333 (0.261–6.818)	
>60	22/23 (95.7%)	1.000 (0.099–10.115)	
<b>Eradication attempts</b>			
Primary	24/25 (96.0%)	1 (Reference)	0.961
Rescue	152/158 (96.2%)	1.056 (0.122–9.156)	
<b>Acid-suppressive drugs</b>			
Rabeprazole	60/65 (92.3%)	1 (Reference)	
Esomeprazole	26/27 (96.3%)	2.167 (0.241–19.472)	0.490
Vonoprazan	90/91 (98.9%)	7.500 (0.855–65.802)	0.069

therapy, and PPI or potassium-competitive acid blocker (P-CAB) use were not significantly associated with an increased risk of eradication failure when guided by *H. pylori* antimicrobial susceptibility testing ( $P > 0.05$ ).

## Discussion

*H. pylori* infection poses an important threat to public health with a high prevalence. Successful eradication of *H. pylori* is crucial for preventing the recurrence of ulcers and occurrence of gastric cancer.<sup>13–15</sup> In recent decades, UBT has been widely used to detect *H. pylori* infection. In this study, we observed that the UBT positivity rate was lower than that reported in a previous study (99.7%).<sup>16</sup> There are several possible explanations for these false-positive results, including an indeterminate UBT result and the growth of urease-producing non-*H. pylori* bacteria. In our study, 8/12 (66.7%) patients were diagnosed with autoimmune gastritis (AIG), characterized by hypochlorhydria and non-*H. pylori* bacteria. Unfortunately, most patients already received more than one course of *H. pylori* eradication. Therefore, gastroscopy should be considered when empirical *H. pylori* eradication fails.

*H. pylori* culture, the gold standard, is the only method that has 100% specificity. However, its sensitivity varies depending on factors including the experience of the laboratory staff, culture methods, and the quality of the sample.<sup>11,17</sup> In our study, the sensitivity of *H. pylori* culture was 89.5%, which is not inferior to that in previous studies.<sup>18</sup> Our successful bacterial culture provided a solid foundation for subsequent sensitivity testing.

The antibiotic resistance of *H. pylori* in the local area showed that the antibiotic resistance rates of MET, CLA, and LEV were high, which was consistent with previously reported results in China.<sup>19,20</sup> The resistance to AMX, FR, and TET remained low, especially for FR and TET. Some studies have reported that *H. pylori* eradication frequency is significantly correlated with the rate of antibiotic resistance.<sup>16,21</sup> Our results showed similar trends for MET, LEV, and AMX but without statistically significant differences. The primary resistance rate of MET was highest. The main reason may be attributed to abuse of MET, which was characterized by availability and affordability. Even if higher doses of MET can overcome its resistance and improve eradication rates, the risk of adverse events will correspondingly increase, especially nausea and vomiting.<sup>22</sup> Moreover, for CLA, the resistance rate was much higher in the non-naive patients. Thus, it is recommended to adopt a cautious approach while prescribing CLA in regions where the resistance rate exceeds 20%. The eradication rates may not achieve satisfactory results for rescue therapy while adopting clarithromycin containing regimens. Therefore, empirical salvage therapy with amoxicillin – or tetracycline- containing 14-d quadruple regimens should be considered when managing patients with multiple treatment failures.<sup>23</sup>

Recent studies have reported an average eradication rate of 80–90% in the bismuth quadruple therapy group.<sup>24,25</sup> In our previous study, the eradication rate of a 14-day quadruple rescue therapy based on amoxicillin and furazolidone was only 80.8% empirically.<sup>26</sup> In cases of amoxicillin allergy, the eradication rate is even lower. The reason might be that most physicians prefer to choose bismuth-based quadruple therapy containing LEV, MET, or CLA, owing to a lack of TET in most Chinese hospitals. However, in our study, guided by AST, the eradication rate significantly improved. Therefore, the AST-guided treatment effectively improved bacterial eradication rates with limited side effects and avoided unnecessary antibiotic use.

Existing evidence suggests that the main determinant of successful *H. pylori* eradication is choosing susceptible antibiotics.<sup>27</sup> In addition to antibiotics, other factors associated with *H. pylori* eradication should also be considered. Previous studies have shown that acid-suppressive drugs play an important role in eradication therapy.<sup>28</sup> The current paradigm favors the concept that new-generation PPIs (esomeprazole or rabeprazole) have a significantly higher eradication rate than first-generation PPIs (omeprazole, lansoprazole, or pantoprazole).<sup>26</sup> Therefore, PPIs including rabeprazole and esomeprazole were chosen for this study. Additionally, vonoprazan, a potassium-competitive acid blocker, was selected by physicians in this retrospective study. In previous studies, vonoprazan was found to be an independent factor for successful eradication in both primary and rescue therapy.<sup>28,29</sup> Although the eradication rate in vonoprazan group was slightly higher than those in new generation PPIs, the difference was not significant. We speculated that the inconsistency was attributed to the small sample size. Large-sample studies need to be conducted in the future.

Our study has several limitations. First, the sample size was relatively small compared with that of other studies. Most patients prefer gastroscopy and *H. pylori* culture when first-line eradication therapy fails. Second, this is a retrospective study. Many patient characteristics were retrieved from electronic medical records without patient compliance data. Third, our study was performed at a single center. These results may not be generalizable to other areas, especially if antibiotic resistance patterns vary geographically. Admittedly, we could not explore potential mutational sites of resistant genes responsible for different antibiotics due to various reasons. There is still a lot of work to be done in the future. Therefore, a prospective multicenter study is required.

In summary, we achieved a high positivity rate for *H. pylori* culture depending on our mature technology. This study demonstrated that MET, CLA, and LEV were the main resistant antibiotics and that there was lower resistance to AMX, FR, and TET in our local outpatients. Based on our experience, we assume that AST-guided therapy can be beneficial for patients in whom first-line therapies fail. If the AST is not available, based on the findings of a high resistance pattern in patients with multiple treatment failures, it may be reasonable to empirically treat with a salvage therapy with amoxicillin – or tetracycline- containing 14-d quadruple regimens.

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## Disclosure

The authors declare no conflict of interest regarding the publication of this paper.

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