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# Short-term outcomes and intermediate-term follow-up of *Helicobacter pylori* infection treatment for naive patients: A retrospective observational study

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## Abstract

**Objectives:** To explore the outcomes of *H. pylori* infection treatments for naive patients in the real-world settings.

**Design:** A retrospective observational study.

**Setting:** Single tertiary level academic hospital in China.

**Participants:** We identified patients receiving initial quadruple therapy for *H. pylori* infection with confirmed status of eradication (n= 23 470) from 2017 to 2020.

**Primary outcome:** Efficacy of different initial *H. pylori* infection treatments.

**Secondary outcome:** Results of urea breath test after *H. pylori* eradication.

**Results:** Among 23 470 patients who received initial *H. pylori* treatment, 21 285 (90.7%) were treated with amoxicillin-based regimens. There was an increment in the number of young patients from 2017 to 2020 (45.0 vs 39.0, $P$<0.0001). The dominant treatments were therapies containing amoxicillin and furazolidone with eradication rate of 87.6% (14 707 / 16 784) and those containing amoxicillin and clarithromycin with eradication rate of 85.5% (3 577 / 4 182). Year, age, antibiotic regimens and the duration of treatments might correlate with the failure of *H. pylori* eradication in a multivariate logistic regression analysis. Lastly, positive urea breath test results after eradication clustered around the cut-off value, which was shown in both $^{13}$C-urea breath test and $^{14}$C-urea breath test.

**Conclusions:** Amoxicillin should be prescribed more commonly and therapies containing amoxicillin and clarithromycin needs to be re-evaluated. Additionally, more attention should be paid to the results of urea breath test after treatment, especially those close to the cut-off value.


**Keywords:** *Helicobacter pylori*; Quadruple therapy; eradication; urea breath test

## Strengths and limitations of this study

- An observational retrospective study based on a large-scale clinical practice.
- All data from Electronic Medical Record System.
- Lack of generality due to limited dataset and data source as a single-center study.
- Inevitable data missing as a retrospective study.

## Introduction

*Helicobacter pylori* (*H. pylori*) is a gram-negative bacterium with prevalence varying from 24.4% to 70.1% worldwide, and accounts for over a third of global infection-attributable cancer cases[1,2]. *H. pylori* infection would result in gastric diseases like chronic active gastritis and peptic ulcer disease, as well as extragastric diseases including heart diseases[3]. As *H. pylori* infection remains a major public health issue, the antibiotic resistance of *H. pylori* increases alarmingly[4]. Fortunately, the reinfection rate stands relatively low[5]. Therefore, the effectiveness of *H. pylori* initial therapy is crucial since the rate of eradication failure accumulates in second or more therapy[6].

Although the prevalence of *H. pylori* infection in mainland China exhibited a slow decline around 0.9% annually in the past decades, it is still at a high level[1,7]. A successful treatment is defined by 90% or higher eradication rate[8]. As the preferred empirical therapy for *H. pylori* infection in China[9], bismuth-containing quadruple therapy achieved an eradication rate of 87.3% in East Asia in a recent meta-analysis[10]. Meanwhile, resistance of *H. pylori* has been increasing in recent years and resistance to clarithromycin is considered as a major cause of the failure of clarithromycin-based therapy[4,11,12]. However, the eradication rate for susceptibility-guided therapy with clarithromycin offers a promising cure rate over 95%[13]. The outcome of clarithromycin-containing therapy in real practice remains uncertain.

Urea breath test (UBT) is a preferred noninvasive method to detect *H. pylori* infection for initial diagnosis and assessment after treatment[14]. It is well acknowledged that results close to cut-off value are not reliable[9]. Setting the cut-off value at a lower level, the sensitivity improves while the specificity maintains a high level[15]. It is suggested that the cut-off value also depends on when to take UBT, that is, before or after the eradication treatment. In most studies, the UBT results in the "grey zone" were not common[16], which seems different from clinical practice. Thus, the discussion on the cut-off value should be back on the front burner.

In this study, we aimed to provide an overview of the management of *H. pylori* infection based on a large-scale clinical practice. This would allow us to visualize the ongoing changes on the diagnosis, treatment and corresponding outcomes of *H. pylori* infection, which may furthermore offer some fresh insights into better management strategies.

## Materials and Methods

### Study design and population

Patients who were diagnosed with *H. pylori* infection and received initial PPI-bismuth-containing quadruple treatments between January 1st 2017 and December 31st 2020 were searched through Electronic Medical Record System of the Second Affiliated Hospital, Zhejiang University School of Medicine (Hangzhou, China). Patients with a positive result for specimen biopsy for *H. pylori* or UBT were diagnosed with *H. pylori* infection. Patients were excluded if they had *H. pylori* eradication treatments before, changed the regimen during therapy, didn't determine the status of *H. pylori* infection after eradication or their clinical data were incomplete. Variables included age, sex, year, prescribed treatment and outcomes. Data extraction was performed in September 2021. Patients' data were deidentified and two researchers checked the data independently.

### Patient and public involvement

Patients and/or the public were not involved in the design, conduct, reporting or dissemination plans of this research.

### Outcomes

Eradication of *H. pylori* infection was confirmed by $^{13}$C-UBT or $^{14}$C-UBT at least 4 weeks after therapy. The cut-off value of $^{13}$C-UBT was 4.0‰ (delta over baseline, DOB), and that of $^{14}$C-UBT was 100 (disintegrations per minute, DPM). Patients were not permitted to take any PPIs 2 weeks prior to the UBT or any antibiotics 4 weeks before the test.

### Statistical analyses

Nonnormally distributed continuous variables are presented as median (IQR) and categorical variables as absolute frequencies (proportions). The primary outcome was eradication rate of *H. pylori* infection. Different first-line treatments were pooled in 7 categories and PPI in 6 (Supplement file 1). Continuous variables were compared using nonparametric tests. Categorical variables were compared using the chi-square test. A

binary logistic regression analysis was performed to examine the relationship between the failure of *H. pylori* eradication and risk factors. In the multivariate analysis, the effect was evaluated by calculating OR and 95% CI. Statistical significance was defined as $p<0.05$ and indicated as asterisks (*). Statistical analyses were performed using *SPSS* software, *version* 26.0 (*SPSS* Inc.) and GraphPad *PRISM* 9.0.

## Results

From January 2017 to December 2020, 25 796 naive patients diagnosed with *H. pylori* infection received PPI-bismuth-containing quadruple therapy and took UBT at least 4 weeks after the treatment. From those, 23 470 (91%) were included in the current analysis (figure 1). Most of them (90.7%, 21 285 / 23 470) were treated with amoxicillin-based regimens.

### Baseline characteristics

The baseline demographic and clinical characteristics are presented in table 1.

**Table 1. Baseline patient characteristics**

| Characteristics | |
|---|---|
| Overall cases | 23470 |
| Age, median (IQR) | 40 (30–54) |
| Sex, N (%) | |
| Male | 11008 (46.9) |
| Female | 12462 (53.1) |
| Year, N (%) | |
| 2017 | 3957 (16.9) |
| 2018 | 6486 (27.6) |
| 2019 | 7568 (32.2) |
| 2020 | 5459 (23.3) |
| Season, N (%) | |
| Spring | 4473 (19.1) |
| Summer | 5942 (25.3) |
| Autumn | 6322 (26.9) |
| Winter | 6733 (28.7) |
| Regimens, N (%) | |
| Amoxicillin + furazolidone | 16784 (71.5) |
| Amoxicillin + clarithromycin | 4182 (17.8) |
| Amoxicillin + levofloxacin | 309 (1.3) |
| Furazolidone + clarithromycin | 1669 (7.1) |
| Furazolidone + levofloxacin | 358 (1.5) |
| Clarithromycin + levofloxacin | 95 (0.4) |
| Others | 73(0.3) |
| Duration, N (%) | |
| 10 | 5641(24.0) |
| 12 | 1060(4.5) |

| | |
|---|---|
| 14 | 16769(71.4) |
| Proton pump inhibitor, N (%) | |
| Rabeprazole10mg | 9654(41.1) |
| Rabeprazole20mg | 21(0.1) |
| Pantoprazole | 5815(24.8) |
| Esomeprazole | 4811(20.5) |
| Omeprazole | 2236(9.5) |
| Lansoprazole | 933(4.0) |

**Time-trend analysis**

Supplement Figure 1A depicts the age distribution among people who received *H. pylori* eradication treatment from 2017 to 2020. We can see there exist bimodal distributions for all the data groups with an increment in the number of young patients along the time. The median age was 45.0 (33.0-54.0) in 2017, 40.0 (31.0-54.0) in 2018, 39.0 (30.0-53.0) in 2019 and 39.0 (30.0-54.0) in 2020 respectively. Supplement Figure 1B shows the proportion of different UBT used, which indicates a growth of $^{14}$C-UBT over time: from 42.2% in 2017 to 59.6% in 2020 ($P$<0.05).

**Efficacy results**

The overall *H. pylori* infection eradication rate rose considerably from 83.8% in 2017 to 86.8% in 2020 (Supplement table 1). Figure 2A shows that amoxicillin-based therapies achieved higher cure rate than amoxicillin-free therapies every year during the time frame (85.5% vs 70.1%, $P$<0.05 in 2017; 88.3% vs 70.8%, $P$<0.05 in 2018; 86.7% vs 77.4%, $P$<0.05 in 2019 and 87.7% vs 75.8%, $P$<0.05 in 2020). Figure 2B indicates the eradication rate of three dominant regimens by year. The eradication rate of therapies containing amoxicillin and furazolidone was higher than that of therapies containing amoxicillin and clarithromycin in 2017 (87.0% vs 78.9%, $P$<0.05). But there is no significant difference between these two therapies afterwards (88.5% vs 86.9%, $P$>0.05 in 2018; 86.7% vs 86.9%, $P$>0.05 in 2019 and 88.1% vs 86.1%, $P$>0.05 in 2020). During the four years, therapies containing furazolidone and clarithromycin had the worst cure rate (63.1% in 2017, 66.3% in 2018, 75.4% in 2019, 75.1% in 2020). The high eradication rate of other therapies might

be inconsistent with the real practice due to the small sample size (Supplement table 2).

The eradication rates were 89.5%, 87.2%, 85.6%, 83.3%, 80.4% in patients aged ≤ 30, 31-40, 41-50, 51-60 and >60 years respectively ($P$<0.001, figure 2C), suggesting a possibly higher eradication rate among younger patients. Additionally, the same age trend could be observed in therapies containing amoxicillin and furazolidone and therapies containing amoxicillin and clarithromycin (figure 2D). In patients aged ≤ 30 and 51-60, therapies containing amoxicillin and furazolidone showed better outcomes than therapies containing amoxicillin and clarithromycin (90.8% vs 87.8%, $P$<0.01 and 85.2% vs 81.8%, $P$<0.05). Elderly patients over 60 years old achieve the lowest cure rate by both kinds of therapies (82.8% vs 78.9%, $P$>0.05).

We also analyzed the role treatment duration and the value of UBT before the treatment played in *H. pylori* eradication (Supplement figure 2). Generally, there is barely any significant difference between 10-day and 14-day therapies with amoxicillin and furazolidone. In therapies with amoxicillin and clarithromycin, 14-day treatment offered a better result than 10-day treatment in 2019 (88.8% vs 82.8%, $P$<0.05), but they are at statistically the same level in 2020 (84.6% vs 88.4%, $P$>0.05). The urea breath test value before the treatment were approximately the same regardless of whether the eradication succeeded ($P$>0.05, Supplement figure 3).

**Multivariate logistic regression analysis on the failure of *H. pylori* eradication**

We used logistic regression model to explore factors predicting the failure of *H. pylori* eradication (Table 2). The multivariate analysis showed that age, year, regimens and treatment duration were associated with the poor outcomes, while sex, season and PPIs were not.

**Table 2. Univariate and multivariate analyses of risk factors for *H. pylori* eradication failure.**

| Characteristics | N | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|---|
| | | OR (95%CI) | *P*-value | OR (95%CI) | *P*-value |
| Age | | | | | |
| ≤ 30 | 5400 | 1.00 | – | 1.00 | – |

| | | | | | |
|---|---|---|---|---|---|
| 30 - 40 | 5634 | 1.25(1.12–1.41) | < 0.001 | 1.25(1.11–1.41) | < 0.001 |
| 40 - 50 | 3774 | 1.43(1.26–1.62) | < 0.001 | 1.43(1.26–1.62) | < 0.001 |
| 50 - 60 | 4310 | 1.68(1.49–1.89) | < 0.001 | 1.70(1.51–1.91) | < 0.001 |
| > 60 | 2586 | 2.04(1.79–2.32) | < 0.001 | 2.01(1.76–2.29) | < 0.001 |
| Sex | | | | Not Selected | |
| Male | 10257 | 1.00 | – | | |
| Female | 11447 | 0.98(0.91–1.06) | 0.646 | | |
| Year, N (%) | | | | | |
| 2017 | 3695 | 1.00 | – | 1.00 | – |
| 2018 | 6123 | 0.82(0.73–0.91) | < 0.001 | 0.85(0.75–0.95) | 0.005 |
| 2019 | 6740 | 0.86(0.77–0.96) | 0.007 | 0.90(0.80–1.01) | 0.071 |
| 2020 | 5146 | 0.80(0.71–0.90) | < 0.001 | 0.86(0.76–0.97) | 0.017 |
| Season, N (%) | | | | Not Selected | |
| Spring | 4008 | 1.00 | – | | |
| Summer | 5489 | 0.86(0.77–0.96) | 0.009 | | |
| Autumn | 5828 | 0.88(0.78–0.98) | 0.021 | | |
| Winter | 6379 | 0.88(0.79–0.98) | 0.024 | | |
| Regimens, N (%) | | | | | |
| Amoxicillin + furazolidone | 16230 | 1.00 | – | 1.00 | – |
| Amoxicillin + clarithromycin | 3885 | 1.19(1.08–1.32) | 0.001 | 1.21(1.09–1.34) | < 0.001 |
| Furazolidone + clarithromycin | 1589 | 2.99(2.66–3.36) | < 0.001 | 2.97(2.64–3.34) | < 0.001 |
| Duration, N (%) | | | | | |
| 10 | 5348 | 1.00 | – | 1.00 | – |
| 14 | 16356 | 0.81(0.75–0.89) | < 0.001 | 0.89(0.82–0.97) | 0.011 |
| Proton pump inhibitor, N (%) | | | | Not Selected | |
| Rabeprazole10mg | 8826 | 1.00 | – | | |
| Rabeprazole20mg | 21 | 1.05(0.31–3.55) | 0.944 | | |
| Pantoprazole | 5455 | 1.11(1.01–1.22) | 0.029 | | |
| Esomeprazole | 4530 | 1.11(1.00–1.23) | 0.049 | | |
| Omeprazole | 2010 | 0.83(0.72–0.96) | 0.014 | | |
| Lansoprazole | 862 | 1.02(0.84–1.25) | 0.818 | | |

Patients who did not receive treatment regimens with amoxicillin plus furazolidone, amoxicillin plus clarithromycin or furazolidone plus clarithromycin were not included in the analyses. Patients who received 12-day treatment were not included in the analyses either. A total of 1766 patients were excluded from the analyses.

**Specificity of urea breath test after *H. pylori* eradication**

Figure 3 plots the results of $^{13}$C-UBT and $^{14}$C-UBT for being positive of naive patients before and after eradication treatment respectively, which demonstrates the consistent distribution characteristics in $^{13}$C-UBT and $^{14}$C-UBT. The median value for patients

considering being positive before eradication treatment was much higher than that after treatment (23.40 (14.30 – 34.90) vs 12.30 (6.50 – 24.60) in $^{13}$C-UBT, $P$<0.0001; 1118.0 (636.0 – 1702.0) vs 303.0 (146.0 – 930.0 in $^{14}$C-UBT, $P$<0.0001). The positive results of UBT for patients after eradication show a cluster around the cut-off value. By contrast, this peculiar feature is absent in the negative results of UBT for patients after eradication (Supplement figure 4).

**Recurrence after confirmation of *H. pylori* eradication with stricter criteria**

Successful eradication with stricter criteria was determined by the UBT at least 8 weeks after the end of initial *H. pylori* eradication treatment[5]. Recurrence was determined by results of UBT higher than 2.5 times the cut-off value after successful eradication. Among 10 056 patients who successfully eradicated *H. pylori*, 1 617 individuals retook the UBT (23 results of theirs was qualitative but not quantitative). The results of 16 in 843 individuals were over 10‰ in $^{13}$C-UBT and 16 in 751 individuals were over 250 in $^{14}$C-UBT (Supplement table 3). The overall recurrence rate was 2.2%. For patients who received amoxicillin-furazolidone regimen and amoxicillin- clarithromycin regimen, the recurrence rate was 1.8% and 2.1% respectively, and there was no significant difference between them.

## Discussion

In this large-scale retrospective study, we present the statistical outcomes and follow-up of initial *H. pylori* treatments throughout a period of 4 years from a single center in East China.

Statement that *H. pylori*-positive individuals should receive early eradication treatment from both personal and social perspectives caused a shift in the former practice concept [17]. Indication for *H. pylori* eradication was also expanded in China, as confirmed *H. pylori* infection is recommended for eradication[9]. We could observe that the age of visitors was decreasing. There were two main clusters, the young and the middle-aged. These two populations faced varied benefits and risks and were treated based on two different strategies accordingly.

From 2017 to 2020, *H. pylori* treatment schemes consist of 21 285 amoxicillin-based regimens and 2 185 amoxicillin-free regimens. The eradication rate of amoxicillin-free treatments was much lower than that of the amoxicillin-based treatments. Amoxicillin is considered as a major component of *H. pylori* treatment for low resistance[4]. Doctors should investigate carefully whether the patients' allergy to penicillin is true. A lot of studies evidenced that most patients who claimed to be allergic to penicillin had negative skin testing in fact[18-20]. What's more, *H. pylori* might correlate with the occurrence and persistence of chronic spontaneous urticaria[21], which might result in false positive skin testing. In addition, some patients mistook adverse reaction like nausea for allergy. Detailed information should be recorded and that would help us identify the truly allergic patients. Furthermore, it is reported that only one case of fatal anaphylaxis might be associated with oral amoxicillin from 1972 to 2007 in UK[22]. De-labeling penicillin allergy is of great concern nowadays and direct challenge might be a safe and effective way[23]. Based on the evidence, we should have more confidence in the safety of oral amoxicillin.

Amoxicillin standard treatment schemes with furazolidone or clarithromycin are most widely used, and both generally prescribed as 14-day regimens. We could see the ongoing

penetration of updated guideline among physicians with hardly few prescriptions including levofloxacin, as levofloxacin is not recommended for initial treatment[9]. *H. pylori* remains highly sensitive to amoxicillin, furazolidone, and tetracycline in China, especially East China[12]. Antibiotic regimens with amoxicillin and furazolidone dominated in the past few years, as tetracycline was not available in our hospital pharmacy. However, furazolidone is not welcomed in some countries despite its low resistance. Federal Drug Agency states WARNING that furazolidone was suspected of damaging fertility or the unborn child[24]. Nevertheless, the International Agency for Research on Cancer classified furazolidone in group 3, unclassifiable as to carcinogenicity in humans[25]. Shire company stopped marketing furazolidone products with voluntarily withdrawal for the concern of little consumption[26]. A meta-analysis declares that 14-day furazolidone-containing regimen with a low daily dose of 200 mg is well-tolerated and moreover, should be a top priority[27]. No serious AEs was reported among the cases in our study. In this way, furazolidone-containing therapies with high eradication rate should be re-evaluated in other countries.

Clarithromycin resistance has boosted in Asia-Pacific region in the past few decades, presumably due to the increasing consumption of macrolides[28-30]. Clarithromycin-containing regimens are not recommended in areas where clarithromycin resistance is over 20%[29]. According to the updated guidelines, gastroenterologists in our hospital were asked to inquire history of prior antibiotic exposure before their prescription. The effectiveness of regimens with amoxicillin and clarithromycin was similar to that of regimens with amoxicillin and furazolidone from 2018 to 2020. This finding suggests that we should take a look at the regimens with clarithromycin again and focus on the potentially effective population. Based on population with high resistance to clarithromycin, metronidazole and levofloxacin, susceptibility-guided therapies and a local proven highly effective empiric regimen both reached optimal level (>95%) of eradication[13]. Thus, the latter one would be a preferred treatment considering its simplicity on this situation. With the controversy of empiric regimen of choice in our region, further prospective studies are warranted for this

scenario.

Factors associated with eradication failure included year, age, antibiotic regimens and the duration of treatments. Patients who received therapies during 2018 to 2020 showed fewer potential possibilities in eradication failure than those in 2017, when the new expert consensus report was published. There might be a relationship between the outcomes and the clinicians' knowledge of clinical practice guideline. And it is illustrated that the older patients were, the more likely the failure of *H. pylori* eradication would happen. However, the "test and treat" strategy for children is not recommended. It is unnecessary until they are middle-school students in Japan or over 14 years old in China[9,14,31]. Thus, a screening among high-school students or undergraduates might be an important measure to improve the eradication rate, reduce the risk of gastric cancer and prevent from transmission to the next generation. It is worth mention that some scholars put forward the opposite view. They observed a lower eradication rate in younger patients, especially those with gastric ulcers [32]. Symptoms and endoscopic and pathological findings might suggest different pathologic mechanisms of *H. pylori* infection. Thus, these factors should be included in following studies to determine the relationship between age and the outcomes of eradication. Consistent with statement that the treatment duration of bismuth quadruple therapy should be extended to 14 days in the Maastricht V/Florence Consensus Report[33], our work showed that there might be a slightly positive correlation between the treatment duration and the outcomes. However, the difference is not significant in the two dominant therapies. This should be further investigated.

In agreement with a prospective study, there might not be an association between the urea breath test value before treatment and the status of *H. pylori* eradication[34]. Patients' outcomes were not significantly altered by different PPIs either. But a meta-analysis showed higher cure rates in new-generation PPIs (esomeprazole and rabeprazole) than first-generation PPIs (omeprazole, lansoprazole and pantoprazole), especially in CYP2C19 extensive metabolisers[35]. Other factors such as adherence to the treatment, cigarette

smoking and genetic factors counted as well[36,37]. These should be explored in further investigations.

UBT is recommended as preferred method for assessments after *H. pylori* eradication, and monoclonal fecal antigen test as an alternative[9]. Incidentally, monoclonal fecal antigen test was not available in our hospital until November 2021. As exempt distribution of a radioactive drug containing one Microcurie of Carbon-14 Urea was approved[38], $^{14}$C-UBT seems to be more frequently used over time, yet less than the predicted considering the economic benefits. Unexpectedly, the results close to the cutoff value were not uncommon. This gives us a new perspective into the results of UBT after eradication treatment. A long-term follow-up of $^{13}$C-UBT results after *H. pylori* eradication suggests that selection of a lower cut-off value may improve diagnostic accuracy for monitoring the *H. pylori* eradication, with hypothesis based on change of the gastric density of microorganisms[15]. Paradoxically, negative UBT results cluster outside the range close to the borderline, while positive ones inside in our cases. This might lead to the misdiagnosis of the eradication failure and an underestimated eradication rate. The stool antigen test, worse still, is reported with less accuracy than the UBT in patients after *H. pylori* eradication with a lower positive predictive value[39,40]. With all these conflicting statements, further studies are needed to address this important but overlooked issue.

Nevertheless, this study has several limitations. Firstly, retrospective studies do not permit any definite conclusions and potential bias is inevitable. Secondly, patient information was incomplete. Factors such as prior antibiotic exposure, resistance to antibiotics, treatment compliance, adherence to treatment, smoking history, the status of *H. pylori* infection among families, socioeconomic status, hygiene status were not included. Thirdly, there are other first-line treatment regimens for *H. pylori* infection[10]. In this study, we only focused on the PPI-bismuth containing therapies, especially amoxicillin-based therapies with furazolidone or clarithromycin. Vonoprazan, a new potent acid inhibitor has been approved for reflux esophagitis yet *H. pylori* infection in China[41]. Vonoprazan-based

therapies achieved over 90% eradication rates, indicating a promising candidate for *H. pylori* infection treatment in the future.

With improved common understanding of *H. pylori*, more public attention might lead to the increasing number of related medical treatment, especially for the young people. Amoxicillin-free regimens account for 9.3% of the treatments. Doctors should be aware of the importance of amoxicillin and correct concept of penicillin allergy. Regimens with amoxicillin and furazolidone dominates among these recorded cases, presumably due to the generally acknowledged rising antibiotic resistance to clarithromycin and levofloxacin in *H. pylori*. However, the observed effectiveness of amoxicillin-clarithromycin containing quadruple therapy shows the otherwise, vacillating the common sense. Furthermore, it is noticeable that the results of both $^{13}$C-UBT and $^{14}$C-UBT taken after *H. pylori* eradication intensively distributes at the threshold level for positivity, which suggests an introspection of the current mainstream diagnostic methods. Further studies to confirm the effectiveness of different regimens and the specificity of UBT in diagnosis are needed.

## STATEMENTS & DECLARATIONS

**Contributions:** All authors contributed to the concept and design of the study. Material preparation, data collection and analysis were performed by YW, YX and OL. The first draft of the manuscript was written by YW and JY. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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**Competing interests:** The authors declare that they have no competing interests.

**Patient consent:** All data were collected with de-identified personal information to ensure that individuals maintained their anonymity. This study was exempted from obtaining individual informed consent as the study was based on routine de-identified data.

**Ethics approval:** The study was approved by the Ethics Committee of the Second Affiliated Hospital, Zhejiang University School of Medicine (registration no. 2021-0716).

**Data availability statement:** Supplementary Data are available at BMJ Open Online.

## References


1. Hooi JKY, Lai WY, Ng WK, et al. Global Prevalence of Helicobacter pylori Infection: Systematic Review and Meta-Analysis. *Gastroenterology.* 2017;153(2):420-429.
2. de Martel C, Georges D, Bray F, Ferlay J, Clifford GM. Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis. *Lancet Glob Health.* 2020;8(2):e180-e190.
3. Robinson K, Atherton JC. The Spectrum of -Mediated Diseases. *Annu Rev Pathol.* 2021;16:123-144.
4. Savoldi A, Carrara E, Graham DY, Conti M, Tacconelli E. Prevalence of Antibiotic Resistance in Helicobacter pylori: A Systematic Review and Meta-analysis in World Health Organization Regions. *Gastroenterology.* Nov 2018;155(5):1372-1382 e1317.
5. Xie Y, Song C, Cheng H, et al. Long-term follow-up of reinfection and its risk factors after initial eradication: a large-scale multicentre, prospective open cohort, observational study. *Emerg Microbes Infect.* 2020;9(1):548-557.
6. Shah SC, Iyer PG, Moss SF. AGA Clinical Practice Update on the Management of Refractory Helicobacter pylori Infection: Expert Review. *Gastroenterology.* Apr 2021;160(5):1831-1841.
7. Li M, Sun Y, Yang J, et al. Time trends and other sources of variation in Helicobacter pylori infection in mainland China: A systematic review and meta-analysis. *Helicobacter.* Oct 2020;25(5):e12729.
8. Graham DY, Lee Y-C, Wu M-S. Rational Helicobacter pylori therapy: evidence-based medicine rather than medicine-based evidence. *Clin Gastroenterol Hepatol.* 2014;12(2).
9. Liu WZ, Xie Y, Lu H, et al. Fifth Chinese National Consensus Report on the management of Helicobacter pylori infection. *Helicobacter.* 2018;23(2):e12475.
10. Rokkas T, Gisbert JP, Malfertheiner P, et al. Comparative Effectiveness of Multiple Different First-Line Treatment Regimens for Helicobacter pylori Infection: A Network Meta-analysis. *Gastroenterology.* 2021;161(2).
11. Thung I, Aramin H, Vavinskaya V, et al. Review article: the global emergence of Helicobacter

pylori antibiotic resistance. *Aliment Pharmacol Ther.* 2016;43(4):514-533.

**12.** Zhong Z, Zhang Z, Wang J, et al. A retrospective study of the antibiotic-resistant phenotypes and genotypes of strains in China. *Am J Cancer Res.* 2021;11(10):5027-5037.

**13.** Chen Q, Long X, Ji Y, et al. Randomised controlled trial: susceptibility-guided therapy versus empiric bismuth quadruple therapy for first-line Helicobacter pylori treatment. *Aliment Pharmacol Ther.* 2019;49(11):1385-1394.

**14.** Kato M, Ota H, Okuda M, et al. Guidelines for the management of Helicobacter pylori infection in Japan: 2016 Revised Edition. *Helicobacter.* 2019;24(4):e12597.

**15.** Gisbert JP, Olivares D, Jimenez I, Pajares JM. Long-term follow-up of 13C-urea breath test results after Helicobacter pylori eradication: frequency and significance of borderline delta13CO2 values. *Aliment Pharmacol Ther.* 2006;23(2):275-280.

**16.** Kato C, Sugiyama T, Sato K, et al. Appropriate cut-off value of 13C-urea breath test after eradication of Helicobacter pylori infection in Japan. *J Gastroenterol Hepatol.* 2003;18(12):1379-1383.

**17.** Sugano K, Tack J, Kuipers EJ, et al. Kyoto global consensus report on Helicobacter pylori gastritis. *Gut.* 2015;64(9):1353-1367.

**18.** Chey WD, Leontiadis GI, Howden CW, Moss SF. ACG Clinical Guideline: Treatment of Helicobacter pylori Infection. *Am J Gastroenterol.* 2017;112(2):212-239.

**19.** Shenoy ES, Macy E, Rowe T, Blumenthal KG. Evaluation and Management of Penicillin Allergy: A Review. *JAMA.* 2019;321(2):188-199.

**20.** Stone CA, Trubiano J, Coleman DT, Rukasin CRF, Phillips EJ. The challenge of de-labeling penicillin allergy. *Allergy.* 2020;75(2):273-288.

**21.** Kim HJ, Kim Y-J, Lee HJ, et al. Systematic review and meta-analysis: Effect of Helicobacter pylori eradication on chronic spontaneous urticaria. *Helicobacter.* 2019;24(6):e12661.

**22.** Lee P, Shanson D. Results of a UK survey of fatal anaphylaxis after oral amoxicillin. *J Antimicrob Chemother.* 2007;60(5):1172-1173.

**23.** Mustafa SS, Conn K, Ramsey A. Comparing Direct Challenge to Penicillin Skin Testing for

the Outpatient Evaluation of Penicillin Allergy: A Randomized Controlled Trial. *J Allergy Clin Immunol Pract.* 2019;7(7):2163-2170.

**24.** https://dailymed.nlm.nih.gov/dailymed/fda/fdaDrugXsl.cfm?setid=056f4352-baaa-4f47-9fb3-fc2bdde6b26e.

**25.** https://monographs.iarc.who.int/list-of-classifications.

**26.** Graham DY, Lu H. Furazolidone in Helicobacter pylori therapy: misunderstood and often unfairly maligned drug told in a story of French bread. *Saudi J Gastroenterol.* 2012;18(1):1-2.

**27.** Ji C-R, Liu J, Li Y-Y, et al. Safety of furazolidone-containing regimen in infection: a systematic review and meta-analysis. *BMJ Open.* 2020;10(10):e037375.

**28.** Megraud F, Bruyndonckx R, Coenen S, et al. resistance to antibiotics in Europe in 2018 and its relationship to antibiotic consumption in the community. *Gut.* 2021;70(10):1815-1822.

**29.** Kuo Y-T, Liou J-M, El-Omar EM, et al. Primary antibiotic resistance in Helicobacter pylori in the Asia-Pacific region: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol.* 2017;2(10):707-715.

**30.** Kocsmár É, Buzás GM, Szirtes I, et al. Primary and secondary clarithromycin resistance in Helicobacter pylori and mathematical modeling of the role of macrolides. *Nat Commun.* 2021;12(1):2255.

**31.** Jones NL, Koletzko S, Goodman K, et al. Joint ESPGHAN/NASPGHAN Guidelines for the Management of Helicobacter pylori in Children and Adolescents (Update 2016). *J Pediatr Gastroenterol Nutr.* 2017;64(6).

**32.** Tang Y, Tang G, Pan L, Zhu H, Zhou S, Wei Z. Clinical factors associated with initial Helicobacter pylori eradication therapy: a retrospective study in China. *Sci Rep.* Sep 21 2020;10(1):15403.

**33.** Malfertheiner P, Megraud F, O'Morain CA, et al. Management of Helicobacter pylori infection-the Maastricht V/Florence Consensus Report. *Gut.* 2017;66(1).

**34.** Gisbert JP, Olivares D, Jimenez I, Pajares JM. Is there any correlation between 13C-urea breath test values and response to first-line and rescue Helicobacter pylori eradication therapies? *Dig*

*Liver Dis.* 2006;38(4):254-259.

**35.** McNicholl AG, Linares PM, Nyssen OP, Calvet X, Gisbert JP. Meta-analysis: esomeprazole or rabeprazole vs. first-generation pump inhibitors in the treatment of Helicobacter pylori infection. *Aliment Pharmacol Ther.* 2012;36(5):414-425.

**36.** Yu J, Yang P, Qin X, Li C, Lv Y, Wang X. Impact of smoking on the eradication of Helicobacter pylori. *Helicobacter.* 2021:e12860.

**37.** Graham DY, Lew GM, Malaty HM, et al. Factors influencing the eradication of Helicobacter pylori with triple therapy. *Gastroenterology.* 1992;102(2):493-496.

**38.** https://www.nrc.gov/reading-rm/doc-collections/cfr/part030/part030-0021.html.

**39.** Bilardi C, Biagini R, Dulbecco P, et al. Stool antigen assay (HpSA) is less reliable than urea breath test for post-treatment diagnosis of Helicobacter pylori infection. *Aliment Pharmacol Ther.* 2002;16(10):1733-1738.

**40.** Perri F, Manes G, Neri M, Vaira D, Nardone G. Helicobacter pylori antigen stool test and 13C-urea breath test in patients after eradication treatments. *Am J Gastroenterol.* 2002;97(11):2756-2762.

**41.** Kagami T, Sahara S, Ichikawa H, et al. Potent acid inhibition by vonoprazan in comparison with esomeprazole, with reference to CYP2C19 genotype. *Aliment Pharmacol Ther.* 2016;43(10):1048-1059.

## Figure Legends

**Figure 1. Study flow chart.**

**Figure 2. Efficacy Results**

(A) The eradication rate of amoxicillin-based regimens and amoxicillin-free regimens. (B) The eradication rate of three dominant therapies by year. (C) The eradication rate by age. (D) The eradication rate of two dominant therapies by age. A, amoxicillin; C, clarithromycin; F, furazolidone.

**Figure 3. Results of UBT for being positive before and after *H. pylori* eradication.**

(A) The scatter plot of $^{13}$C-UBT for being positive before and after *H. pylori* eradication. (B) The scatter plot of $^{14}$C-UBT for being positive before and after *H. pylori* eradication. The cut-off value of $^{13}$C-UBT was 4.0‰ (delta over baseline, DOB), and that of $^{14}$C-UBT was 100 (disintegrations per minute, DPM).

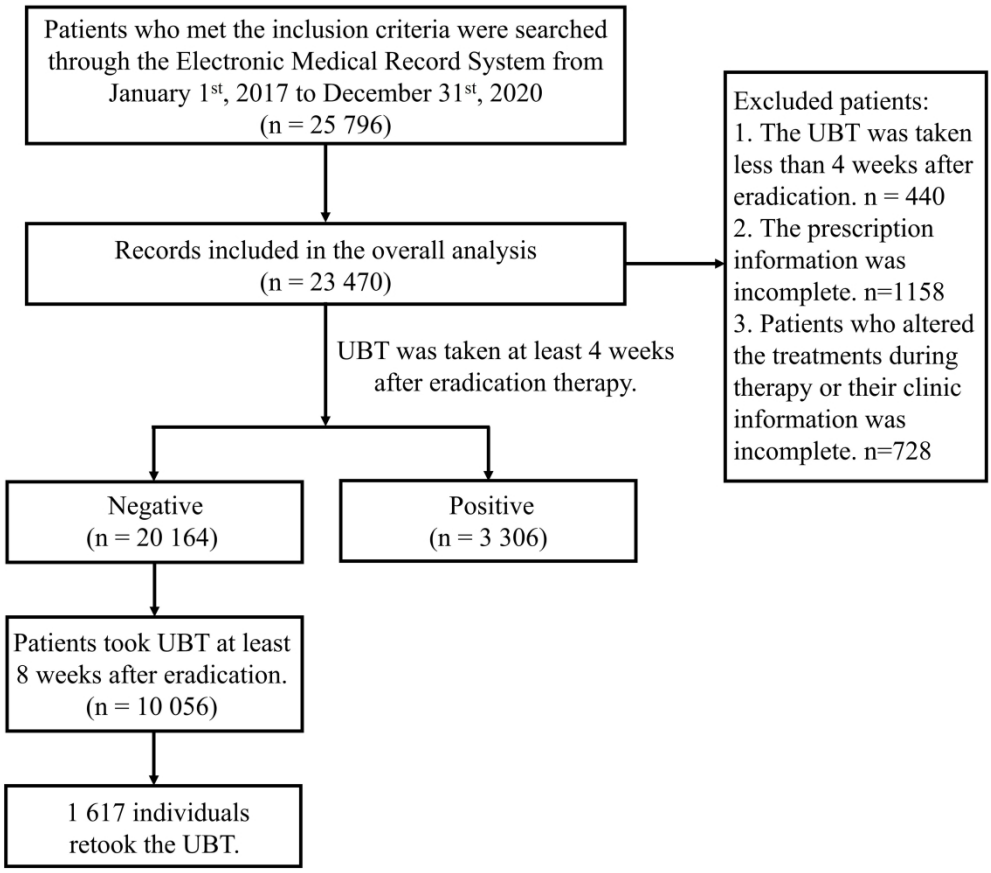



Figure 1. Study flow chart.

319x278mm (300 x 300 DPI)

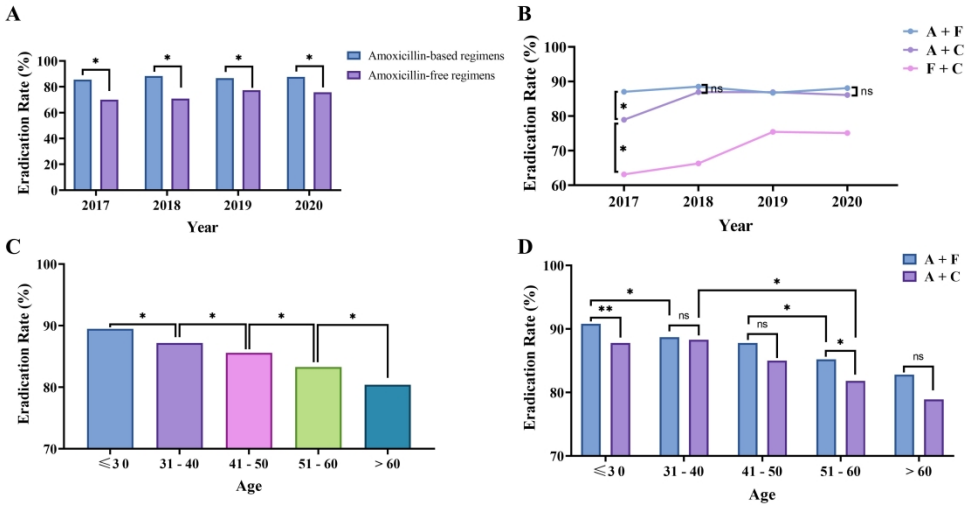



Figure 2. Efficacy Results
(A) The eradication rate of amoxicillin-based regimens and amoxicillin-free regimens. (B) The eradication rate of three dominant therapies by year. (C) The eradication rate by age. (D) The eradication rate of two dominant therapies by age. A, amoxicillin; C, clarithromycin; F, furazolidone.

387x201mm (300 x 300 DPI)

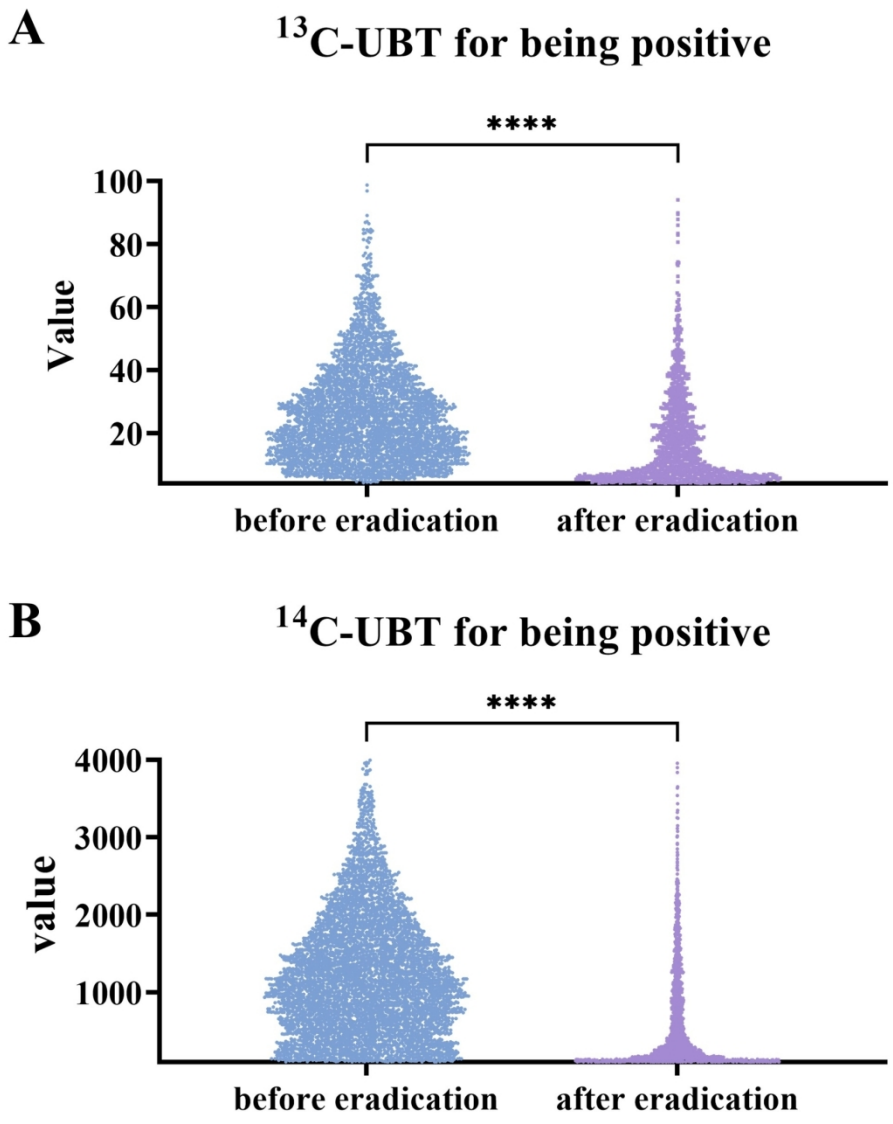



Figure 3. Results of UBT for being positive before and after H. pylori eradication.
(A) The scatter plot of 13C-UBT for being positive before and after H. pylori eradication. (B) The scatter plot of 14C-UBT for being positive before and after H. pylori eradication. The cut-off value of 13C-UBT was 4.0‰ (delta over baseline, DOB), and that of 14C-UBT was 100 (disintegrations per minute, DPM).

165x191mm (300 x 300 DPI)

# ONLINE SUPPLEMENTARY MATERIAL

## Supplementary File 1. The original protocol for the study

Data were searched through Electronic Medical Record System of the Second Affiliated Hospital, Zhejiang University School of Medicine (Hangzhou, China). Data extraction was performed in September 2021 by IT department.

Inclusion criteria:

1. time frame (from January 1st 2017 to December 31st 2020)
2. diagnosed with *H. pylori* infection
3. received initial PPI-bismuth-containing quadruple treatments
4. with the results of urea breath test at least 4 weeks after eradication therapy

Exclusion criteria:

1. had *H. pylori* eradication treatments before
2. changed the regimen during therapy
3. clinical data were incomplete

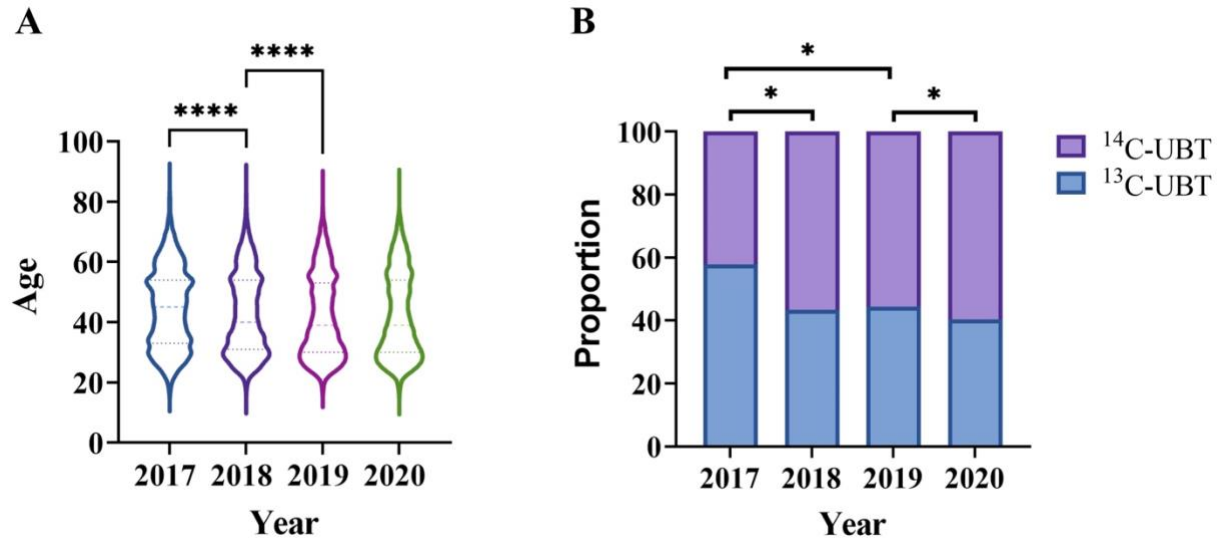
Patients' data were deidentified and two researchers checked the data independently. Variables included age, sex, year, prescribed treatment and outcomes.

# Supplementary File 2. First-line Bismuth-containing quadruple therapy for *H. pylori* infection category pools and Proton pump inhibitor categories

1. PPI + bismuth + A + F
2. PPI + bismuth + A + C
3. PPI + bismuth + F + C
4. PPI + bismuth + A + L
5. PPI + bismuth + F + L
6. PPI + bismuth + C + L
7. Other regimens (including different first-line therapies with frequencies lower than 0.5%)

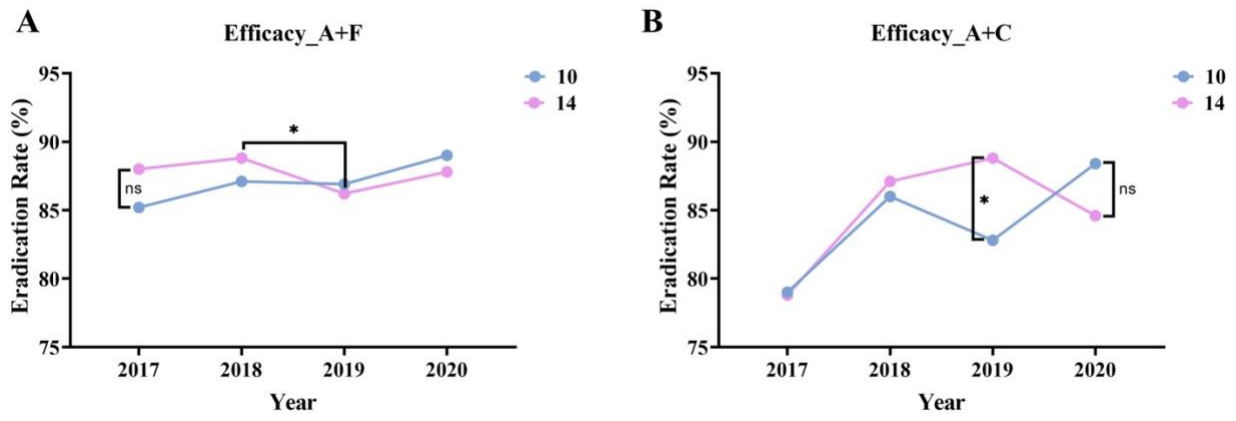
Standard dose PPI including rabeprazole 10 mg (or 20 mg), pantoprazole 40 mg, esomeprazole 20 mg, omeprazole 20 mg and lansoprazole 30mg. Bismuth-containing quadruple therapy is defined as a PPI together with two antibiotics and bismuth salts given in the standard way. A, amoxicillin; C, clarithromycin; F, furazolidone; L, levofloxacin.

**Supplement Figure 1. Temporal trend analysis (2017–2020).**



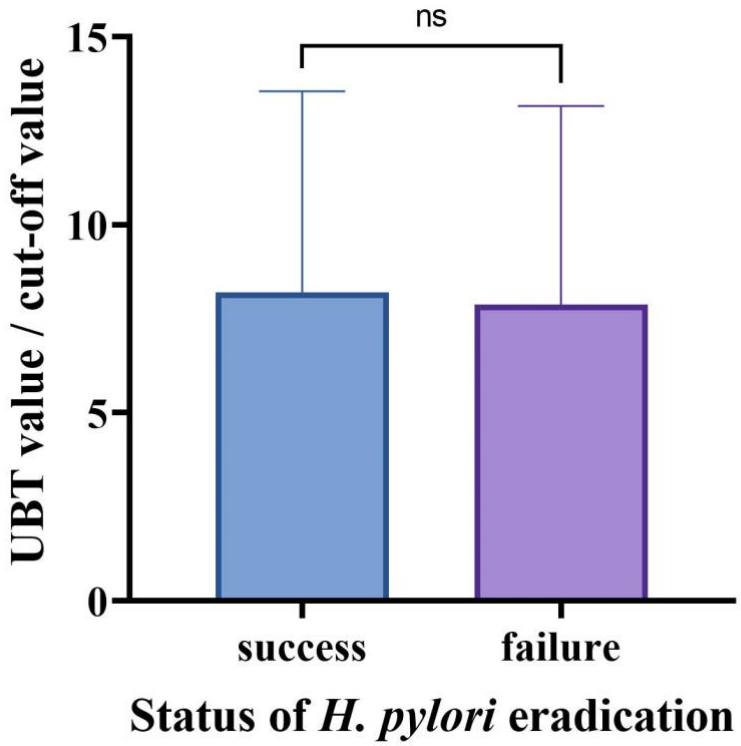

(A) Trends in the age of visitors. (B) Trends in the assessments after treatments.

**Supplement Figure 2. Efficacy Results by treatment duration.**



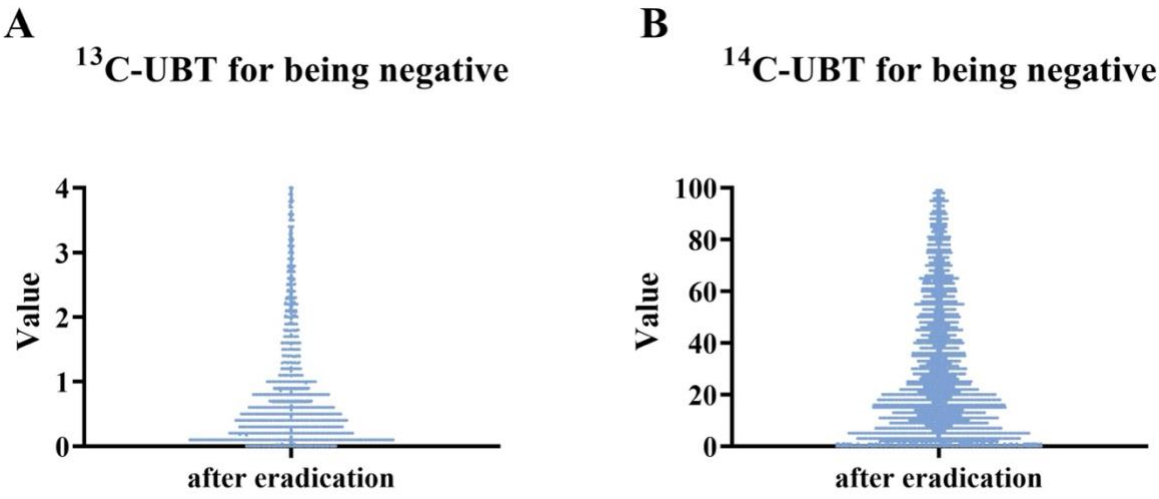

(A) The eradication rate of regimens with amoxicillin and furazolidone. (B) The eradication rate of regimens with amoxicillin and clarithromycin.

**Supplement Figure 3. The association between UBT value before treatments and the outcomes.**




Data is standardized by dividing the UBT value by the cut-off value in different tests. The cut-off value of $^{13}$C-UBT was 4.0‰ (delta over baseline, DOB), and that of $^{14}$C-UBT was 100 (disintegrations per minute, DPM).

**Supplement Figure 4. Results of UBT for being negative after *H. pylori* eradication.**




(A) The scatter plot of $^{13}$C-UBT after *H. pylori* eradication. (B) The scatter plot of $^{14}$C-UBT after *H. pylori* eradication. The cut-off value of $^{13}$C-UBT was 4.0‰ (delta over baseline, DOB), and that of $^{14}$C-UBT was 100 (disintegrations per minute, DPM).

**Supplement Table 1. Effectiveness of first-line treatments per year.**

| Year | N | Success | Eradication rate (%) |
|---|---|---|---|
| 2017 | 3957 | 3317 | 83.8% |
| 2018 | 6486 | 5605 | 86.4%* |
| 2019 | 7568 | 6506 | 86.0%* |
| 2020 | 5459 | 4736 | 86.8%* |

* $P<0.05$ vs 2017.

**Supplement Table 2. Effectiveness of different first-line treatments per year.**

| Year | Regimen | N | Success | Eradication rate (%) |
|---|---|---|---|---|
| 2017 | A + F | 2785 | $2424_{a}$ | 87.0% |
| | A + C | 639 | $504_{b}$ | 78.9% |
| | F + C | 279 | $176_{c}$ | 63.1% |
| | A + L | 97 | $83_{a, b}$ | 85.6% |
| | F + L | 109 | $95_{a, b}$ | 87.2% |
| | C + L | 28 | $15_{c}$ | 53.6% |
| | Others | 20 | $20_{a, b}$ | 100.0% |
| 2018 | A + F | 4830 | $4275_{a}$ | 88.5% |
| | A + C | 863 | $750_{a}$ | 86.9% |
| | F + C | 498 | $330_{b, c}$ | 66.3% |
| | A + L | 110 | $96_{a}$ | 87.3% |
| | F + L | 122 | $107_{a}$ | 87.7% |
| | C + L | 22 | $11_{c}$ | 50.0% |
| | Others | 41 | $36_{a, b}$ | 87.8% |
| 2019 | A + F | 5009 | $4344_{a}$ | 86.7% |
| | A + C | 1853 | $1611_{a}$ | 86.9% |
| | F + C | 495 | $373_{b}$ | 75.4% |
| | A + L | 81 | $67_{a, b}$ | 82.7% |
| | F + L | 93 | $86_{a}$ | 92.5% |
| | C + L | 27 | $17_{b}$ | 63.0% |
| | Others | 10 | $8_{a, b}$ | 80.0% |
| 2020 | A + F | 4160 | $3664_{a}$ | 88.1% |
| | A + C | 827 | $712_{a}$ | 86.1% |
| | F + C | 397 | $298_{b}$ | 75.1% |
| | A + L | 21 | $18_{a, b}$ | 85.7% |
| | F + L | 34 | $30_{a, b}$ | 88.2% |
| | C + L | 18 | $13_{a, b}$ | 72.2% |
| | Others | 2 | $1_{a, b}$ | 50.0% |

Each subscript letter (a or b) denotes a subset of year categories whose column proportions do not differ significantly from each other at the .05 level. A, amoxicillin; C, clarithromycin; F, furazolidone; L, levofloxacin.

**Supplement Table 3. Recurrence after confirmation of H. pylori eradication with stricter criteria.**

| | Test | N | Standardized positivity | Recurrence rate |
|---|---|---|---|---|
| overall | overall | 1457 | 32 | 2.2% |
| | $^{13}$C-UBT | 843 | 16 | |
| | $^{14}$C-UBT | 751 | 16 | |
| A+F | overall | 1192 | 21 | 1.8% |
| | $^{13}$C-UBT | 636 | 9 | |
| | $^{14}$C-UBT | 556 | 12 | |
| A+C | overall | 242 | 5 | 2.1% |
| | $^{13}$C-UBT | 126 | 4 | |
| | $^{14}$C-UBT | 116 | 1 | |

Standardized positivity was defined by over 10‰ in $^{13}$C-UBT and over 250 in $^{14}$C-UBT. A, amoxicillin; C, clarithromycin; F, furazolidone.

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

| | Item No | Recommendation | Page No |
|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found | 1, 2 |
| **Introduction** | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 4, 5 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 5 |
| **Methods** | | | |
| Study design | 4 | Present key elements of study design early in the paper | 6 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 6 |
| Participants | 6 | (*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up | 6 |
| | | (*b*) For matched studies, give matching criteria and number of exposed and unexposed | Not applicable |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 6 |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 6 |
| Bias | 9 | Describe any efforts to address potential sources of bias | 6 |
| Study size | 10 | Explain how the study size was arrived at | 6 |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 6,7 |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding | 6,7 |
| | | (*b*) Describe any methods used to examine subgroups and interactions | 6,7 |
| | | (*c*) Explain how missing data were addressed | 6 |
| | | (*d*) If applicable, explain how loss to follow-up was addressed | 6 |
| | | (*e*) Describe any sensitivity analyses | Not applicable |
| **Results** | | | |
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | 8 |
| | | (b) Give reasons for non-participation at each stage | 8 |
| | | (c) Consider use of a flow diagram | 8 |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | 8 |
| | | (b) Indicate number of participants with missing data for each variable of interest | 8 |
| | | (c) Summarise follow-up time (eg, average and total amount) | 8 |
| Outcome data | 15* | Report numbers of outcome events or summary measures over time | 8 |

| | | | |
|---|---|---|---|
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included<br>(*b*) Report category boundaries when continuous variables were categorized<br>(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | 8, 9<br>8, 9<br>Not applicable |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | 9, 10 |
| **Discussion** | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 15 |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 14 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 11, 12, 13, 14 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | 14, 15 |
| **Other information** | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 16 |

*Give information separately for exposed and unexposed groups.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

BMJ Open

# Short-term outcomes and intermediate-term follow-up of *Helicobacter pylori* infection treatment for naive patients: A retrospective observational study

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| Secondary Subject Heading: | Gastroenterology and hepatology |
| Keywords: | Gastroduodenal disease < GASTROENTEROLOGY, Gastrointestinal infections < GASTROENTEROLOGY, INFECTIOUS DISEASES, Diagnostic microbiology < INFECTIOUS DISEASES |
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# Short-term outcomes and intermediate-term follow-up of *Helicobacter pylori* infection treatment for naive patients: A retrospective observational study

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## Abstract

**Objectives:** To explore the outcomes of *H. pylori* infection treatments for naive patients in the real-world settings.

**Design:** A retrospective observational study.

**Setting:** Single tertiary level academic hospital in China.

**Participants:** We identified patients receiving initial quadruple therapy for *H. pylori* infection with confirmed status of eradication (n= 23 470) from 2017 to 2020.

**Primary outcome:** Efficacy of different initial *H. pylori* infection treatments.

**Secondary outcome:** Results of urea breath test after *H. pylori* eradication.

**Results:** Among 23 470 patients who received initial *H. pylori* treatment, 21 285 (90.7%) were treated with amoxicillin-based regimens. The median age of the patients was decreasing from 2017 to 2020 (45.0 vs 39.0, $P$<0.0001). The dominant treatments were therapies containing amoxicillin and furazolidone with eradication rate of 87.6% (14 707 / 16 784) and those containing amoxicillin and clarithromycin with eradication rate of 85.5% (3 577 / 4 182). Date of treatment, age, antibiotic regimens and the duration of treatments showed correlation with the failure of *H. pylori* eradication in a multivariable logistic regression analysis. Lastly, positive urea breath test results after eradication clustered around the cut-off value, which was shown in both $^{13}$C-urea breath test and $^{14}$C-urea breath test.

**Conclusions:** The dominant *H. pylori* infection treatments for naive patients were therapies containing amoxicillin and furazolidone, which offered a highest eradication rate. Date of treatment, age, antibiotic regimens and the duration of treatments were risk factors for the failure of *H. pylori* eradication. Additionally, positive urea breath test results after eradication clustered around the cut-off value.


**Keywords:** *Helicobacter pylori*; Quadruple therapy; eradication; urea breath test

## Strengths and limitations of this study

- This observational retrospective study is based on a large-scale clinical practice to avoid data bias and improve the comprehensiveness.
- All data comes from Electronic Medical Record System, which ensures the authenticity and relatively high completeness.
- Lack of generality due to limited dataset and data source as this is a single-center study.
- Inevitable data missing in this retrospective study as the treatment protocol cannot be strictly enforced.

## Introduction

*Helicobacter pylori* (*H. pylori*) is a gram-negative bacterium with prevalence varying from 24.4% to 70.1% worldwide, and accounts for over a third of global infection-attributable cancer cases [1,2]. *H. pylori* infection results in gastric diseases like chronic active gastritis and peptic ulcer disease, as well as extragastric diseases including heart diseases [3]. As *H. pylori* infection remains a major public health issue, the antibiotic resistance of *H. pylori* increases alarmingly [4]. Fortunately, the reinfection rate stands relatively low [5]. Therefore, the effectiveness of initial *H. pylori* therapy is crucial since the rate of eradication failure accumulates in second or more therapy [6].

Although the prevalence of *H. pylori* infection in mainland China exhibited a slow decline around 0.9% annually in the past decades, it is still at a high level [1,7]. A successful treatment is defined by 90% or higher eradication rate [8]. As the preferred empirical therapy for *H. pylori* infection in China [9], bismuth-containing quadruple therapy achieved an eradication rate of 87.3% in East Asia in a recent meta-analysis [10]. Meanwhile, resistance of *H. pylori* has been increasing in recent years and resistance to clarithromycin is considered as a major cause of the failure of clarithromycin-based therapy [4,11,12]. However, the eradication rate for susceptibility-guided therapy with clarithromycin offers a promising cure rate over 95% [13]. The outcome of clarithromycin-containing therapy in real practice remains uncertain.

Urea breath test (UBT) is a preferred noninvasive method to detect *H. pylori* infection for initial diagnosis and assessment after treatment [14]. The principle of UBT is based on the highly active urease enzymes produced by *H. pylori*, which catalyzes the reaction of labelled urea molecule into labelled carbon dioxide that can be detected in breath samples [15]. $^{13}$C-UBT and $^{14}$C-UBT showed similar sensitivity and specificity [16]. $^{13}$C-UBT can be used in children and pregnant women, while $^{14}$C-UBT is not allowed among those populations for its radioactivity [17]. It is widely acknowledged that results close to cut-off value are not reliable [9]. Setting the cut-off value at a lower level, the sensitivity improves

while the specificity maintains a high level [18]. It is suggested that the cut-off value also depends on when to take UBT, that is, before or after the eradication treatment. In most studies, the UBT results in the "grey zone" were not common [19], which seems different from clinical practices. Thus, considerations about the cut-off value for UBT after *H. pylori* eradication should still be evaluated in the light of new evidence.

In this study, we aimed to provide an overview of the management of *H. pylori* infection based on a large-scale clinical practice. This would allow us to visualize the ongoing changes on the diagnosis, treatment, and corresponding outcomes of *H. pylori* infection, which may furthermore offer some fresh insights into better management strategies.

## Materials and Methods

### Study design and population

Patients who were diagnosed with *H. pylori* infection and received initial proton pump inhibitor (PPI)-bismuth-containing quadruple treatments between January 1st 2017 and December 31st 2020 were searched through Electronic Medical Record System of the Second Affiliated Hospital, Zhejiang University School of Medicine (Hangzhou, China). Patients with a positive result for specimen biopsy for *H. pylori* or UBT were diagnosed with *H. pylori* infection. Patients were excluded if they had *H. pylori* eradication treatments before, changed the regimen during therapy, didn't determine the status of *H. pylori* infection after eradication, or their clinical data were incomplete. Variables included age, sex, date of treatment, prescribed treatment and outcomes. Data extraction was performed in September 2021. Patients' data were deidentified and two researchers checked the data independently.

### Patient and public involvement

Patients and/or the public were not involved in the design, conduct, reporting or dissemination plans of this research.

### Follow-up and Outcomes

Follow-up was performed by outpatient clinical visits. Patients were asked to revisit the outpatient clinics at least 4 weeks after completion of *H. pylori* therapy. Eradication of *H. pylori* infection was confirmed by $^{13}$C-UBT or $^{14}$C-UBT at least 4 weeks after therapy. The cut-off value of $^{13}$C-UBT was 4.0‰ (delta over baseline, DOB), and that of $^{14}$C-UBT was 100 (disintegrations per minute, DPM). Patients were not permitted to take any PPIs 2 weeks prior to the UBT or any antibiotics 4 weeks before the test.

### Univariate and multivariable logistic analyses

A binary logistic regression analysis was performed to examine the relationship between the failure of *H. pylori* eradication and risk factors. In the multivariable analysis, the effect was evaluated by calculating odds ratios (OR) and 95% confidence intervals (95%

CI). Patients who did not receive treatment regimens with amoxicillin plus furazolidone, amoxicillin plus clarithromycin or furazolidone plus clarithromycin were not included in the analyses. Patients who received 12-day treatment were not included in the analyses either. In total, 1766 patients were excluded from the analyses.

**Statistical analyses**

Nonnormally distributed continuous variables are presented as median (IQR) and categorical variables as absolute frequencies (proportions). The primary outcome was eradication rate of *H. pylori* infection. Different first-line treatments were pooled in 7 categories and PPI in 6 (Supplement file 1). Continuous variables were compared using nonparametric test (Kruskal-Wallis test). Categorical variables were compared using the chi-square test. Statistical significance was defined as $P<0.05$ and indicated as asterisks (*). Statistical analyses were performed using *SPSS* software, *version* 26.0 (*SPSS* Inc.) and GraphPad *PRISM* 9.0.

## Results

From January 2017 to December 2020, 25 796 naive patients diagnosed with *H. pylori* infection received PPI-bismuth-containing quadruple therapy and took UBT at least 4 weeks after the treatment. From those, 23 470 (91%) were included in the current analysis (figure 1). Most of them (90.7%, 21 285 / 23 470) were treated with amoxicillin-based regimens.

### Baseline characteristics

The baseline demographic and clinical characteristics are presented in table 1.

**Table 1. Baseline patient characteristics**

| Characteristics | |
|---|---|
| Overall cases | 23470 |
| Age, median (IQR) | 40 (30–54) |
| Sex, N (%) | |
| Male | 11008 (46.9) |
| Female | 12462 (53.1) |
| Date of treatment, N (%) | |
| 2017 | 3957 (16.9) |
| 2018 | 6486 (27.6) |
| 2019 | 7568 (32.2) |
| 2020 | 5459 (23.3) |
| Season, N (%) | |
| Spring | 4473 (19.1) |
| Summer | 5942 (25.3) |
| Autumn | 6322 (26.9) |
| Winter | 6733 (28.7) |
| Antibiotic regimens, N (%) | |
| Amoxicillin + furazolidone | 16784 (71.5) |
| Amoxicillin + clarithromycin | 4182 (17.8) |
| Amoxicillin + levofloxacin | 309 (1.3) |
| Furazolidone + clarithromycin | 1669 (7.1) |
| Furazolidone + levofloxacin | 358 (1.5) |
| Clarithromycin + levofloxacin | 95 (0.4) |
| Others | 73(0.3) |
| Duration, N (%) | |
| 10 | 5641(24.0) |
| 12 | 1060(4.5) |

| | |
|---|---|
| 14 | 16769(71.4) |
| Proton pump inhibitor, N (%) | |
| Rabeprazole10mg | 9654(41.1) |
| Rabeprazole20mg | 21(0.1) |
| Pantoprazole | 5815(24.8) |
| Esomeprazole | 4811(20.5) |
| Omeprazole | 2236(9.5) |
| Lansoprazole | 933(4.0) |

**Time-trend analysis**

Supplement Figure 1A depicts the age distribution among people who received *H. pylori* eradication treatment from 2017 to 2020. We can see there exist bimodal distributions for all the data groups with an increment in the number of young patients along the time. The median age was 45.0 (33.0-54.0) in 2017, 40.0 (31.0-54.0) in 2018, 39.0 (30.0-53.0) in 2019 and 39.0 (30.0-54.0) in 2020 respectively. Supplement Figure 1B shows the proportion of different UBT used, which indicates a growth of $^{14}$C-UBT over time: from 42.2% in 2017 to 59.6% in 2020 ($P$<0.05).

**Efficacy results**

The overall *H. pylori* infection eradication rate rose considerably from 83.8% in 2017 to 86.8% in 2020 (Supplement table 1). Figure 2A shows that amoxicillin-based therapies achieved higher cure rate than amoxicillin-free therapies every year during the time frame (85.5% vs 70.1%, $P$<0.05 in 2017; 88.3% vs 70.8%, $P$<0.05 in 2018; 86.7% vs 77.4%, $P$<0.05 in 2019 and 87.7% vs 75.8%, $P$<0.05 in 2020). Figure 2B indicates the eradication rate of three dominant regimens by date of treatment. The eradication rate of therapies containing amoxicillin and furazolidone was higher than that of therapies containing amoxicillin and clarithromycin in 2017 (87.0% vs 78.9%, $P$<0.05). But there is no significant difference between these two therapies afterwards (88.5% vs 86.9%, $P$>0.05 in 2018; 86.7% vs 86.9%, $P$>0.05 in 2019 and 88.1% vs 86.1%, $P$>0.05 in 2020). During the four years, therapies containing furazolidone and clarithromycin had the lowest cure rate (63.1% in 2017, 66.3% in 2018, 75.4% in 2019, 75.1% in 2020). The high eradication rate

of other therapies might be inconsistent with the real practice due to the small sample size (Supplement table 2).

The eradication rates were 89.5%, 87.2%, 85.6%, 83.3%, 80.4% in patients aged ≤ 30, 31-40, 41-50, 51-60 and >60 years respectively ($P$<0.001, figure 2C), suggesting a possibly higher eradication rate among younger patients. Additionally, the same age trend could be observed in therapies containing amoxicillin and furazolidone and therapies containing amoxicillin and clarithromycin (figure 2D). In patients aged ≤ 30 and 51-60, therapies containing amoxicillin and furazolidone showed better outcomes than therapies containing amoxicillin and clarithromycin (90.8% vs 87.8%, $P$<0.01 and 85.2% vs 81.8%, $P$<0.05). Elderly patients over 60 years old achieve the lowest cure rate by both kinds of therapies (82.8% vs 78.9%, $P$>0.05).

We also analyzed how the treatment duration and the value of UBT before the treatment impacted the *H. pylori* eradication (Supplement figure 2). Generally, there is barely any significant difference between 10-day and 14-day therapies with amoxicillin and furazolidone. In therapies with amoxicillin and clarithromycin, 14-day treatment offered a better result than 10-day treatment in 2019 (88.8% vs 82.8%, $P$<0.05), but they are at statistically the same level in 2020 (84.6% vs 88.4%, $P$>0.05). The urea breath test value before the treatment were approximately the same regardless of whether the eradication succeeded ($P$>0.05, Supplement figure 3).

**Multivariable logistic regression analysis on the failure of *H. pylori* eradication**

We used a logistic regression model to explore factors predicting the failure of *H. pylori* eradication (Table 2). The multivariable analysis showed that age, date of treatment, antibiotic regimens and treatment duration were associated with the poor outcomes, while sex, season and PPIs were not.

**Table 2. Univariate and multivariable analyses of risk factors for *H. pylori* eradication failure.**

| Characteristics | N | Univariate analysis | | Multivariable analysis | |
|---|---|---|---|---|---|
| | | OR (95%CI) | *P*-value | OR (95%CI) | *P*-value |

| | | | | | |
|---|---|---|---|---|---|
| Age | | | | | |
| ≤ 30 | 5400 | 1.00 | – | 1.00 | – |
| 30 - 40 | 5634 | 1.25(1.12–1.41) | < 0.001 | 1.25(1.11–1.41) | < 0.001 |
| 40 - 50 | 3774 | 1.43(1.26–1.62) | < 0.001 | 1.43(1.26–1.62) | < 0.001 |
| 50 - 60 | 4310 | 1.68(1.49–1.89) | < 0.001 | 1.70(1.51–1.91) | < 0.001 |
| > 60 | 2586 | 2.04(1.79–2.32) | < 0.001 | 2.01(1.76–2.29) | < 0.001 |
| Sex | | | | | |
| Male | 10257 | 1.00 | – | | |
| Female | 11447 | 0.98(0.91–1.06) | 0.646 | | |
| Date of treatment | | | | | |
| 2017 | 3695 | 1.00 | – | 1.00 | – |
| 2018 | 6123 | 0.82(0.73–0.91) | < 0.001 | 0.85(0.75–0.95) | 0.005 |
| 2019 | 6740 | 0.86(0.77–0.96) | 0.007 | 0.90(0.80–1.01) | 0.071 |
| 2020 | 5146 | 0.80(0.71–0.90) | < 0.001 | 0.86(0.76–0.97) | 0.017 |
| Season | | | | | |
| Spring | 4008 | 1.00 | – | | |
| Summer | 5489 | 0.86(0.77–0.96) | 0.009 | | |
| Autumn | 5828 | 0.88(0.78–0.98) | 0.021 | | |
| Winter | 6379 | 0.88(0.79–0.98) | 0.024 | | |
| Antibiotic regimens | | | | | |
| Amoxicillin + furazolidone | 16230 | 1.00 | – | 1.00 | – |
| Amoxicillin + clarithromycin | 3885 | 1.19(1.08–1.32) | 0.001 | 1.21(1.09–1.34) | < 0.001 |
| Furazolidone + clarithromycin | 1589 | 2.99(2.66–3.36) | < 0.001 | 2.97(2.64–3.34) | < 0.001 |
| Duration | | | | | |
| 10 | 5348 | 1.00 | – | 1.00 | – |
| 14 | 16356 | 0.81(0.75–0.89) | < 0.001 | 0.89(0.82–0.97) | 0.011 |
| Proton pump inhibitor | | | | | |
| Rabeprazole10mg | 8826 | 1.00 | – | | |
| Rabeprazole20mg | 21 | 1.05(0.31–3.55) | 0.944 | | |
| Pantoprazole | 5455 | 1.11(1.01–1.22) | 0.029 | | |
| Esomeprazole | 4530 | 1.11(1.00–1.23) | 0.049 | | |
| Omeprazole | 2010 | 0.83(0.72–0.96) | 0.014 | | |
| Lansoprazole | 862 | 1.02(0.84–1.25) | 0.818 | | |

**Specificity of urea breath test after *H. pylori* eradication**

Figure 3 plots the results of $^{13}$C-UBT and $^{14}$C-UBT for being positive of naive patients before and after eradication treatment respectively, which demonstrates the consistent distribution characteristics in $^{13}$C-UBT and $^{14}$C-UBT. The median value for patients considering being positive before eradication treatment was much higher than that after

treatment (23.40 (14.30 – 34.90) vs 12.30 (6.50 – 24.60) in $^{13}$C-UBT, $P$<0.0001; 1118.0 (636.0 – 1702.0) vs 303.0 (146.0 – 930.0) in $^{14}$C-UBT, $P$<0.0001). The positive results of UBT for patients after eradication show a cluster around the cut-off value. By contrast, this peculiar feature is absent in the negative results of UBT for patients after eradication (Supplement figure 4).

**Recurrence after confirmation of *H. pylori* eradication with stricter criteria**

Successful eradication with stricter criteria was determined by the UBT at least 8 weeks after the end of initial *H. pylori* eradication treatment[5]. Recurrence was determined by results of UBT higher than 2.5 times the cut-off value after successful eradication. Among 10 056 patients who successfully eradicated *H. pylori*, 1 617 individuals retook the UBT (23 results of theirs was qualitative but not quantitative). The results of 16 in 843 individuals were over 10‰ in $^{13}$C-UBT and 16 in 751 individuals were over 250 in $^{14}$C-UBT (Supplement table 3). The overall recurrence rate was 2.2%. For patients who received amoxicillin-furazolidone regimen and amoxicillin- clarithromycin regimen, the recurrence rate was 1.8% and 2.1% respectively, and there was no significant difference between them.

## Discussion

In this large-scale retrospective study, we presented the statistical outcomes and follow-up of initial *H. pylori* treatments throughout a period of 4 years from a single center in East China.

Statement that *H. pylori*-positive individuals should receive early eradication treatment from both personal and social perspectives caused a shift in the former practice concept [20]. Indication for *H. pylori* eradication was also expanded in China, as confirmed *H. pylori* infection is recommended for eradication [9]. We could observe that the age of visitors was decreasing. There were two main clusters, the young and the middle-aged. These two populations faced varied benefits and risks and were treated based on two different strategies accordingly.

From 2017 to 2020, *H. pylori* treatment schemes consist of 21 285 amoxicillin-based regimens and 2 185 amoxicillin-free regimens. The eradication rate of amoxicillin-free treatments was much lower than that of the amoxicillin-based treatments. Amoxicillin is considered as a major component of *H. pylori* treatment for low resistance [4]. Doctors should investigate carefully whether the patients' allergy to penicillin is true. A lot of studies evidenced that most patients who claimed to be allergic to penicillin had negative skin testing in fact [21-23]. What's more, *H. pylori* might correlate with the occurrence and persistence of chronic spontaneous urticaria [24], which might result in false positive skin testing. In addition, some patients mistook adverse reaction like nausea for allergy. Detailed information should be recorded and that would help us identify the truly allergic patients. Furthermore, it is reported that only one case of fatal anaphylaxis might be associated with oral amoxicillin from 1972 to 2007 in UK [25]. De-labeling penicillin allergy is of great concern nowadays and direct challenge might be a safe and effective way [26]. Based on the evidence, we should have more confidence in the safety of oral amoxicillin.

Amoxicillin standard treatment schemes with furazolidone or clarithromycin are most widely used, and both generally prescribed as 14-day regimens. We could see the ongoing

penetration of updated guideline among physicians with hardly few prescriptions including levofloxacin, as levofloxacin is not recommended for initial treatment [9]. *H. pylori* remains highly sensitive to amoxicillin, furazolidone, and tetracycline in China, especially East China[12]. Antibiotic regimens with amoxicillin and furazolidone dominated in the past few years, as tetracycline was not available in our hospital pharmacy. However, furazolidone is not welcomed in some countries despite its low resistance. Federal Drug Agency states WARNING that furazolidone was suspected of damaging fertility or the unborn child [27]. Nevertheless, the International Agency for Research on Cancer classified furazolidone in group 3, unclassifiable as to carcinogenicity in humans [28]. Shire company stopped marketing furazolidone products with voluntarily withdrawal for the concern of little consumption [29]. A meta-analysis declares that 14-day furazolidone-containing regimen with a low daily dose of 200 mg is well-tolerated and moreover, should be a top priority [30]. No serious AEs was reported among the cases in our study. In this way, furazolidone-containing therapies with high eradication rate should be re-evaluated in other countries.

Clarithromycin resistance has boosted in Asia-Pacific region in the past few decades, presumably due to the increasing consumption of macrolides [31-33]. Clarithromycin-containing regimens are not recommended in areas where clarithromycin resistance is over 20% [32]. However, the effectiveness of regimens with amoxicillin and clarithromycin was similar to that of regimens with amoxicillin and furazolidone from 2018 to 2020. According to the updated guidelines, gastroenterologists in our hospital were asked to inquire history of prior antibiotic exposure before their prescription, which might be a possible explanation for the contradiction. This finding suggests that we should further investigate regimens with clarithromycin for *H. pylori* eradication and focus on the potentially effective population. Based on population with high resistance to clarithromycin, metronidazole and levofloxacin, susceptibility-guided therapies and a local proven highly effective empiric regimen both reached optimal level (>95%) of eradication [13]. Thus, the latter one would be a preferred treatment considering its simplicity on this

situation. With the controversy of empiric regimen of choice in our region, further prospective studies are warranted for this scenario.

Factors associated with eradication failure included date of treatment, age, antibiotic regimens and the duration of treatments. Patients who received therapies during 2018 to 2020 showed fewer potential possibilities in eradication failure than those in 2017, when the new expert consensus report was published. There might be a relationship between the outcomes and the clinicians' knowledge of clinical practice guideline. And it is illustrated that the older patients were, the more likely the failure of *H. pylori* eradication would happen. However, the "test and treat" strategy for children is not recommended. It is unnecessary until they are middle-school students in Japan or over 14 years old in China [9,14,34]. Thus, a screening among high-school students or undergraduates might be an important measure to improve the eradication rate, reduce the risk of gastric cancer and prevent from transmission to the next generation. It is worth mention that some scholars put forward the opposite view. They observed a lower eradication rate in younger patients, especially those with gastric ulcers [35]. Symptoms and endoscopic and pathological findings might suggest different pathologic mechanisms of *H. pylori* infection. Thus, these factors should be included in following studies to determine the relationship between age and the outcomes of eradication. Consistent with statement that the treatment duration of bismuth quadruple therapy should be extended to 14 days in the Maastricht V/Florence Consensus Report [17], our work showed that there was a slightly positive correlation between the treatment duration and the outcomes. However, the difference is not significant in the two dominant therapies. This should be further investigated.

In agreement with a prospective study, there was not an association between the urea breath test value before treatment and the status of *H. pylori* eradication [36]. Patients' outcomes were not significantly altered by different PPIs either. But a meta-analysis showed higher cure rates in new-generation PPIs (esomeprazole and rabeprazole) than first-generation PPIs (omeprazole, lansoprazole and pantoprazole), especially in CYP2C19

extensive metabolizers [37]. Other factors such as adherence to the treatment, cigarette smoking and genetic factors counted as well [38,39]. These should be explored in further investigations.

UBT is recommended as preferred method for assessments after *H. pylori* eradication, and monoclonal fecal antigen test as an alternative [9]. Incidentally, monoclonal fecal antigen test was not available in our hospital until November 2021. As exempt distribution of a radioactive drug containing one Microcurie of Carbon-14 Urea was approved [40], $^{14}$C-UBT was more frequently used over time, yet less than the predicted considering the economic benefits. Unexpectedly, the results close to the cutoff value were not uncommon. This gives us a new perspective into the results of UBT after eradication treatment. A long-term follow-up of $^{13}$C-UBT results after *H. pylori* eradication suggests that selection of a lower cut-off value may improve diagnostic accuracy for monitoring the *H. pylori* eradication, with hypothesis based on change of the gastric density of microorganisms [18]. Paradoxically, negative UBT results cluster outside the range close to the borderline, while positive ones inside in our cases. This might lead to the misdiagnosis of the eradication failure and an underestimated eradication rate. The stool antigen test, worse still, is reported with less accuracy than the UBT in patients after *H. pylori* eradication with a lower positive predictive value [41,42]. With all these conflicting statements, further studies are needed to address this important but overlooked issue.

Nevertheless, this study has several limitations. Firstly, retrospective studies do not permit any definite conclusions and potential bias is inevitable. Follow-up was not scheduled as strict as a prospective protocol due to the retrospective nature of the study. Secondly, patient information was incomplete. Factors such as prior antibiotic exposure, resistance to antibiotics, treatment compliance, adherence to treatment, smoking history, the status of *H. pylori* infection among families, socioeconomic status, hygiene status were not included. Thirdly, there are other first-line treatment regimens for *H. pylori* infection [10]. In this study, we only focused on the PPI-bismuth containing therapies, especially

amoxicillin-based therapies with furazolidone or clarithromycin. Vonoprazan, a new potent acid inhibitor has been approved for reflux esophagitis yet *H. pylori* infection in China [43]. Vonoprazan-based therapies achieved over 90% eradication rates, indicating a promising candidate for *H. pylori* infection treatment in the future.

With improved common understanding of *H. pylori*, more public attention might lead to the increasing number of related medical treatment, especially for the young people. Amoxicillin-free regimens accounted for 9.3% of the treatments. Doctors should be aware of the importance of amoxicillin and correct concept of penicillin allergy. Regimens with amoxicillin and furazolidone dominated among these recorded cases, presumably due to the generally acknowledged rising antibiotic resistance to clarithromycin and levofloxacin in *H. pylori*. However, the observed effectiveness of amoxicillin-clarithromycin containing quadruple therapy shows the otherwise, vacillating the common sense. Furthermore, it is noticeable that the results of both $^{13}$C-UBT and $^{14}$C-UBT taken after *H. pylori* eradication intensively distributed at the threshold level for positivity, which suggests an introspection of the current mainstream diagnostic methods. Further studies to confirm the effectiveness of different regimens and the specificity of UBT in diagnosis are needed.

## STATEMENTS & DECLARATIONS

**Contributions:** Conception and design: Du Q, Ye J and Wang Y. Acquisition of data: Wang Y, Xiang Y, Liao O, Wu Y and Li Y. Analysis or interpretation of the data: Wang Y, Xiang Y and Ye J. Drafting of the manuscript: Wang Y and Ye J. Revision of the manuscript: Ye J, Du Q and Li Y. Study guarantor and supervision: Du Q and Ye J.

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**Competing interests:** The authors declare that they have no competing interests.

**Patient consent:** All data were collected with de-identified personal information to ensure that individuals maintained their anonymity. This study was exempted from obtaining individual informed consent as the study was based on routine de-identified data.

**Ethics approval:** The study was approved by the Ethics Committee of the Second Affiliated Hospital, Zhejiang University School of Medicine (registration no. 2021-0716).

**Data availability statement:** Data are available from the corresponding author upon reasonable request.

## References


1. Hooi JKY, Lai WY, Ng WK, et al. Global Prevalence of Helicobacter pylori Infection: Systematic Review and Meta-Analysis. *Gastroenterology.* 2017;153(2):420-429.
2. de Martel C, Georges D, Bray F, Ferlay J, Clifford GM. Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis. *The Lancet. Global health.* 2020;8(2):e180-e190.
3. Robinson K, Atherton JC. The Spectrum of -Mediated Diseases. *Annual review of pathology.* 2021;16:123-144.
4. Savoldi A, Carrara E, Graham DY, Conti M, Tacconelli E. Prevalence of Antibiotic Resistance in Helicobacter pylori: A Systematic Review and Meta-analysis in World Health Organization Regions. *Gastroenterology.* Nov 2018;155(5):1372-1382 e1317.
5. Xie Y, Song C, Cheng H, et al. Long-term follow-up of reinfection and its risk factors after initial eradication: a large-scale multicentre, prospective open cohort, observational study. *Emerging microbes & infections.* 2020;9(1):548-557.
6. Shah SC, Iyer PG, Moss SF. AGA Clinical Practice Update on the Management of Refractory Helicobacter pylori Infection: Expert Review. *Gastroenterology.* Apr 2021;160(5):1831-1841.
7. Li M, Sun Y, Yang J, et al. Time trends and other sources of variation in Helicobacter pylori infection in mainland China: A systematic review and meta-analysis. *Helicobacter.* Oct 2020;25(5):e12729.
8. Graham DY, Lee Y-C, Wu M-S. Rational Helicobacter pylori therapy: evidence-based medicine rather than medicine-based evidence. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association.* 2014;12(2).
9. Liu WZ, Xie Y, Lu H, et al. Fifth Chinese National Consensus Report on the management of Helicobacter pylori infection. *Helicobacter.* 2018;23(2):e12475.
10. Rokkas T, Gisbert JP, Malfertheiner P, et al. Comparative Effectiveness of Multiple Different First-Line Treatment Regimens for Helicobacter pylori Infection: A Network Meta-analysis. *Gastroenterology.* 2021;161(2).
11. Thung I, Aramin H, Vavinskaya V, et al. Review article: the global emergence of Helicobacter pylori antibiotic resistance. *Alimentary pharmacology & therapeutics.* 2016;43(4):514-533.
12. Zhong Z, Zhang Z, Wang J, et al. A retrospective study of the antibiotic-resistant phenotypes and genotypes of strains in China. *American journal of cancer research.* 2021;11(10):5027-5037.
13. Chen Q, Long X, Ji Y, et al. Randomised controlled trial: susceptibility-guided therapy versus empiric bismuth quadruple therapy for first-line Helicobacter pylori treatment. *Alimentary pharmacology & therapeutics.* 2019;49(11):1385-1394.
14. Kato M, Ota H, Okuda M, et al. Guidelines for the management of Helicobacter pylori infection in Japan: 2016 Revised Edition. *Helicobacter.* 2019;24(4):e12597.
15. Keller J, Hammer HF, Afolabi PR, et al. European guideline on indications, performance and clinical impact of (13) C-breath tests in adult and pediatric patients: An EAGEN, ESNM, and ESPGHAN consensus, supported by EPC. *United European Gastroenterol J.* Jun 2021;9(5):598-625.
16. Ferwana M, Abdulmajeed I, Alhajiahmed A, et al. Accuracy of urea breath test in Helicobacter

pylori infection: meta-analysis. *World J Gastroenterol.* Jan 28 2015;21(4):1305-1314.

**17.** Malfertheiner P, Megraud F, O'Morain CA, et al. Management of Helicobacter pylori infection-the Maastricht V/Florence Consensus Report. *Gut.* 2017;66(1).

**18.** Gisbert JP, Olivares D, Jimenez I, Pajares JM. Long-term follow-up of 13C-urea breath test results after Helicobacter pylori eradication: frequency and significance of borderline delta13CO2 values. *Alimentary pharmacology & therapeutics.* 2006;23(2):275-280.

**19.** Kato C, Sugiyama T, Sato K, et al. Appropriate cut-off value of 13C-urea breath test after eradication of Helicobacter pylori infection in Japan. *Journal of gastroenterology and hepatology.* 2003;18(12):1379-1383.

**20.** Sugano K, Tack J, Kuipers EJ, et al. Kyoto global consensus report on Helicobacter pylori gastritis. *Gut.* 2015;64(9):1353-1367.

**21.** Chey WD, Leontiadis GI, Howden CW, Moss SF. ACG Clinical Guideline: Treatment of Helicobacter pylori Infection. *The American journal of gastroenterology.* 2017;112(2):212-239.

**22.** Shenoy ES, Macy E, Rowe T, Blumenthal KG. Evaluation and Management of Penicillin Allergy: A Review. *JAMA.* 2019;321(2):188-199.

**23.** Stone CA, Trubiano J, Coleman DT, Rukasin CRF, Phillips EJ. The challenge of de-labeling penicillin allergy. *Allergy.* 2020;75(2):273-288.

**24.** Kim HJ, Kim Y-J, Lee HJ, et al. Systematic review and meta-analysis: Effect of Helicobacter pylori eradication on chronic spontaneous urticaria. *Helicobacter.* 2019;24(6):e12661.

**25.** Lee P, Shanson D. Results of a UK survey of fatal anaphylaxis after oral amoxicillin. *The Journal of antimicrobial chemotherapy.* 2007;60(5):1172-1173.

**26.** Mustafa SS, Conn K, Ramsey A. Comparing Direct Challenge to Penicillin Skin Testing for the Outpatient Evaluation of Penicillin Allergy: A Randomized Controlled Trial. *The journal of allergy and clinical immunology. In practice.* 2019;7(7):2163-2170.

**27.** https://dailymed.nlm.nih.gov/dailymed/fda/fdaDrugXsl.cfm?setid=056f4352-baaa-4f47-9fb3-fc2bdde6b26e.

**28.** https://monographs.iarc.who.int/list-of-classifications.

**29.** Graham DY, Lu H. Furazolidone in Helicobacter pylori therapy: misunderstood and often unfairly maligned drug told in a story of French bread. *Saudi journal of gastroenterology : official journal of the Saudi Gastroenterology Association.* 2012;18(1):1-2.

**30.** Ji C-R, Liu J, Li Y-Y, et al. Safety of furazolidone-containing regimen in infection: a systematic review and meta-analysis. *BMJ open.* 2020;10(10):e037375.

**31.** Megraud F, Bruyndonckx R, Coenen S, et al. resistance to antibiotics in Europe in 2018 and its relationship to antibiotic consumption in the community. *Gut.* 2021;70(10):1815-1822.

**32.** Kuo Y-T, Liou J-M, El-Omar EM, et al. Primary antibiotic resistance in Helicobacter pylori in the Asia-Pacific region: a systematic review and meta-analysis. *The lancet. Gastroenterology & hepatology.* 2017;2(10):707-715.

**33.** Kocsmár É, Buzás GM, Szirtes I, et al. Primary and secondary clarithromycin resistance in Helicobacter pylori and mathematical modeling of the role of macrolides. *Nature communications.* 2021;12(1):2255.

**34.** Jones NL, Koletzko S, Goodman K, et al. Joint ESPGHAN/NASPGHAN Guidelines for the

Management of Helicobacter pylori in Children and Adolescents (Update 2016). *Journal of pediatric gastroenterology and nutrition.* 2017;64(6).

**35.** Tang Y, Tang G, Pan L, Zhu H, Zhou S, Wei Z. Clinical factors associated with initial Helicobacter pylori eradication therapy: a retrospective study in China. *Sci Rep.* Sep 21 2020;10(1):15403.

**36.** Gisbert JP, Olivares D, Jimenez I, Pajares JM. Is there any correlation between 13C-urea breath test values and response to first-line and rescue Helicobacter pylori eradication therapies? *Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver.* 2006;38(4):254-259.

**37.** McNicholl AG, Linares PM, Nyssen OP, Calvet X, Gisbert JP. Meta-analysis: esomeprazole or rabeprazole vs. first-generation pump inhibitors in the treatment of Helicobacter pylori infection. *Alimentary pharmacology & therapeutics.* 2012;36(5):414-425.

**38.** Yu J, Yang P, Qin X, Li C, Lv Y, Wang X. Impact of smoking on the eradication of Helicobacter pylori. *Helicobacter.* 2021:e12860.

**39.** Graham DY, Lew GM, Malaty HM, et al. Factors influencing the eradication of Helicobacter pylori with triple therapy. *Gastroenterology.* 1992;102(2):493-496.

**40.** https://www.nrc.gov/reading-rm/doc-collections/cfr/part030/part030-0021.html.

**41.** Bilardi C, Biagini R, Dulbecco P, et al. Stool antigen assay (HpSA) is less reliable than urea breath test for post-treatment diagnosis of Helicobacter pylori infection. *Alimentary pharmacology & therapeutics.* 2002;16(10):1733-1738.

**42.** Perri F, Manes G, Neri M, Vaira D, Nardone G. Helicobacter pylori antigen stool test and 13C-urea breath test in patients after eradication treatments. *The American journal of gastroenterology.* 2002;97(11):2756-2762.

**43.** Kagami T, Sahara S, Ichikawa H, et al. Potent acid inhibition by vonoprazan in comparison with esomeprazole, with reference to CYP2C19 genotype. *Alimentary pharmacology & therapeutics.* 2016;43(10):1048-1059.

## Figure Legends

**Figure 1. Study flow chart.**

**Figure 2. Efficacy Results**

(A) The eradication rate of amoxicillin-based regimens and amoxicillin-free regimens. (B) The eradication rate of three dominant therapies by date of treatment. (C) The eradication rate by age. (D) The eradication rate of two dominant therapies by age. A, amoxicillin; C, clarithromycin; F, furazolidone.

**Figure 3. Results of UBT for being positive before and after *H. pylori* eradication.**

(A) The scatter plot of $^{13}$C-UBT for being positive before and after *H. pylori* eradication. (B) The scatter plot of $^{14}$C-UBT for being positive before and after *H. pylori* eradication. The cut-off value of $^{13}$C-UBT was 4.0‰ (delta over baseline, DOB), and that of $^{14}$C-UBT was 100 (disintegrations per minute, DPM).

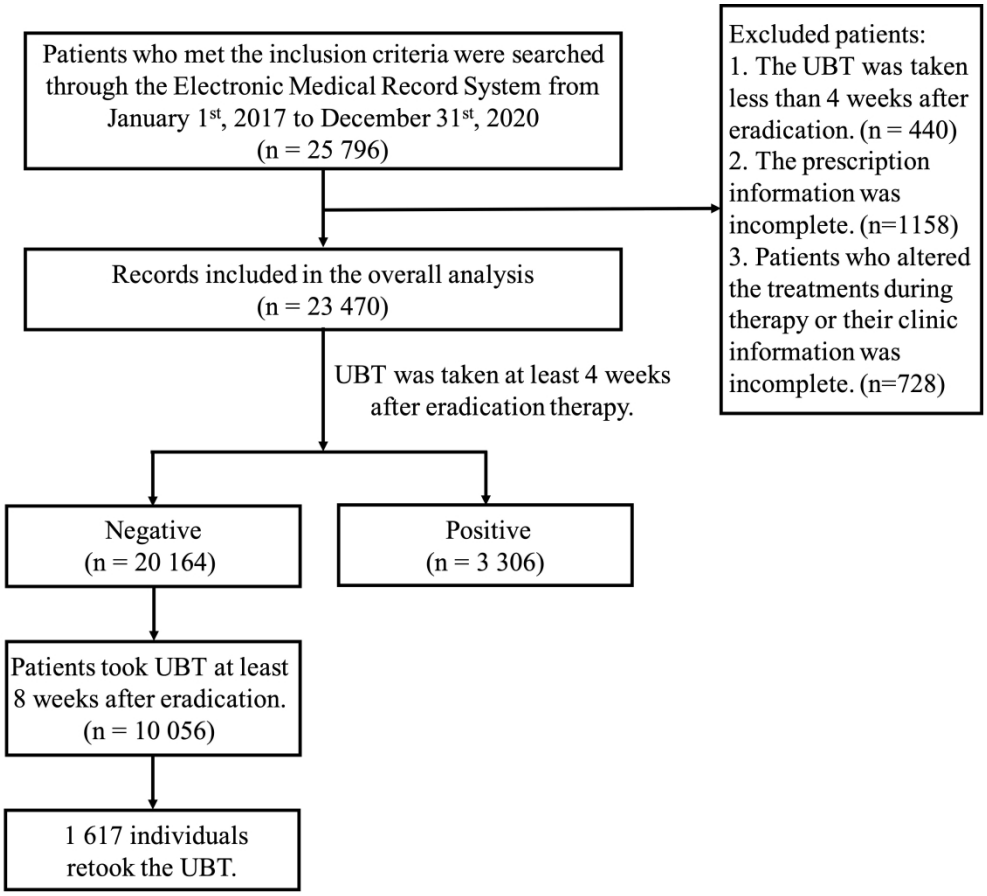



Figure 1. Study flow chart.

1334x1193mm (72 x 72 DPI)

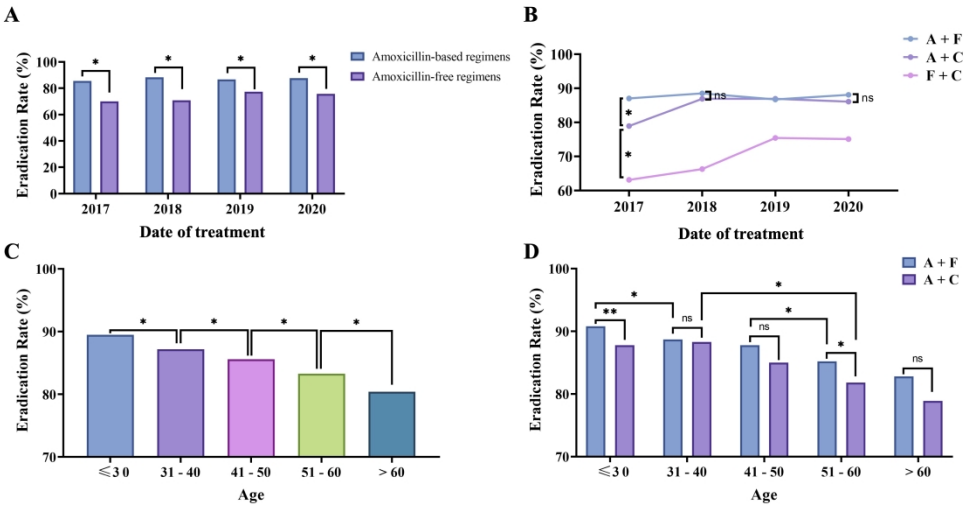



Figure 2. Efficacy Results
(A) The eradication rate of amoxicillin-based regimens and amoxicillin-free regimens. (B) The eradication rate of three dominant therapies by date of treatment. (C) The eradication rate by age. (D) The eradication rate of two dominant therapies by age. A, amoxicillin; C, clarithromycin; F, furazolidone.

1590x824mm (72 x 72 DPI)

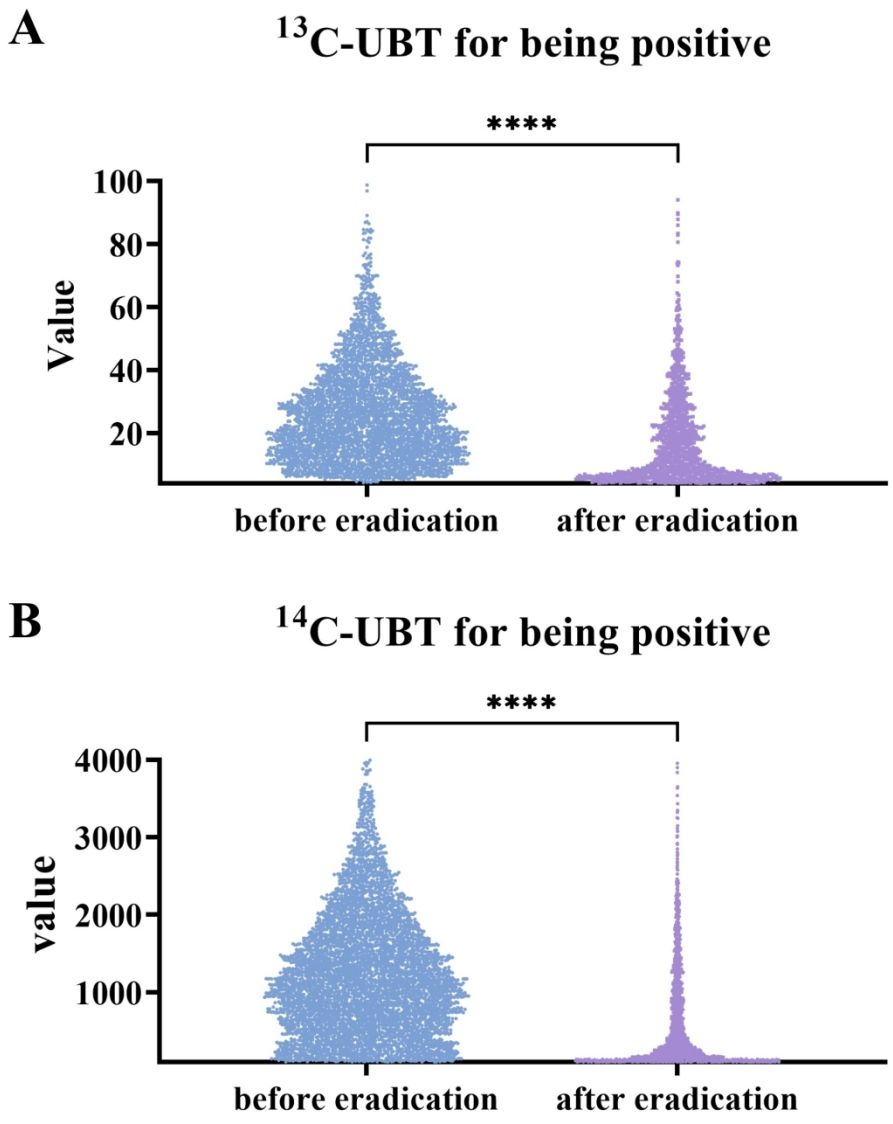



Figure 3. Results of UBT for being positive before and after H. pylori eradication.
(A) The scatter plot of 13C-UBT for being positive before and after H. pylori eradication. (B) The scatter plot of 14C-UBT for being positive before and after H. pylori eradication. The cut-off value of 13C-UBT was 4.0‰ (delta over baseline, DOB), and that of 14C-UBT was 100 (disintegrations per minute, DPM).

165x191mm (330 x 330 DPI)

# ONLINE SUPPLEMENTARY MATERIAL

## Supplementary File 1. The original protocol for the study

Data were searched through Electronic Medical Record System of the Second Affiliated Hospital, Zhejiang University School of Medicine (Hangzhou, China). Data extraction was performed in September 2021 by IT department.

Inclusion criteria:

1. time frame (from January 1st 2017 to December 31st 2020)
2. diagnosed with *H. pylori* infection
3. received initial PPI-bismuth-containing quadruple treatments
4. with the results of urea breath test at least 4 weeks after eradication therapy

Exclusion criteria:

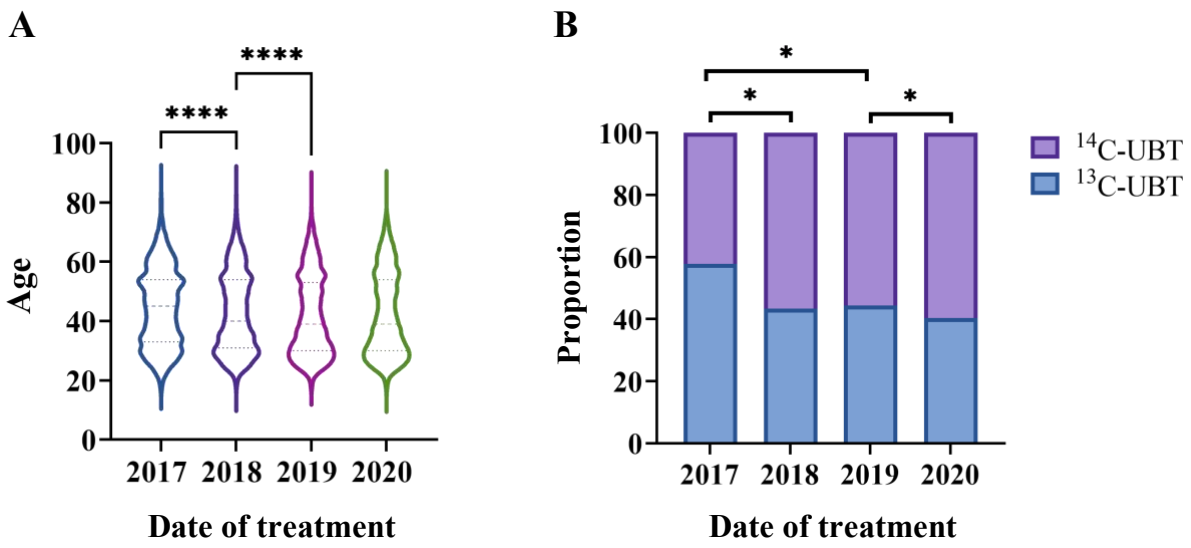
1. had *H. pylori* eradication treatments before
2. changed the regimen during therapy
3. clinical data were incomplete

Patients' data were deidentified and two researchers checked the data independently. Variables included age, sex, year, prescribed treatment and outcomes.

# Supplementary File 2. First-line Bismuth-containing quadruple therapy for *H. pylori* infection category pools and Proton pump inhibitor categories

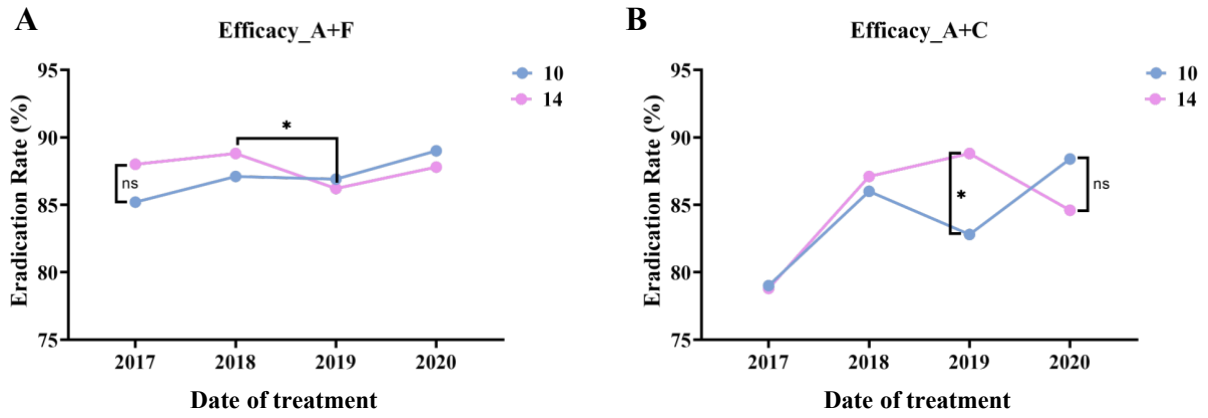
1. PPI + bismuth + A + F
2. PPI + bismuth + A + C
3. PPI + bismuth + F + C
4. PPI + bismuth + A + L
5. PPI + bismuth + F + L
6. PPI + bismuth + C + L
7. Other regimens (including different first-line therapies with frequencies lower than 0.5%)

Standard dose PPI including rabeprazole 10 mg (or 20 mg), pantoprazole 40 mg, esomeprazole 20 mg, omeprazole 20 mg and lansoprazole 30mg. Bismuth-containing quadruple therapy is defined as a PPI together with two antibiotics and bismuth salts given in the standard way. A, amoxicillin; C, clarithromycin; F, furazolidone; L, levofloxacin.



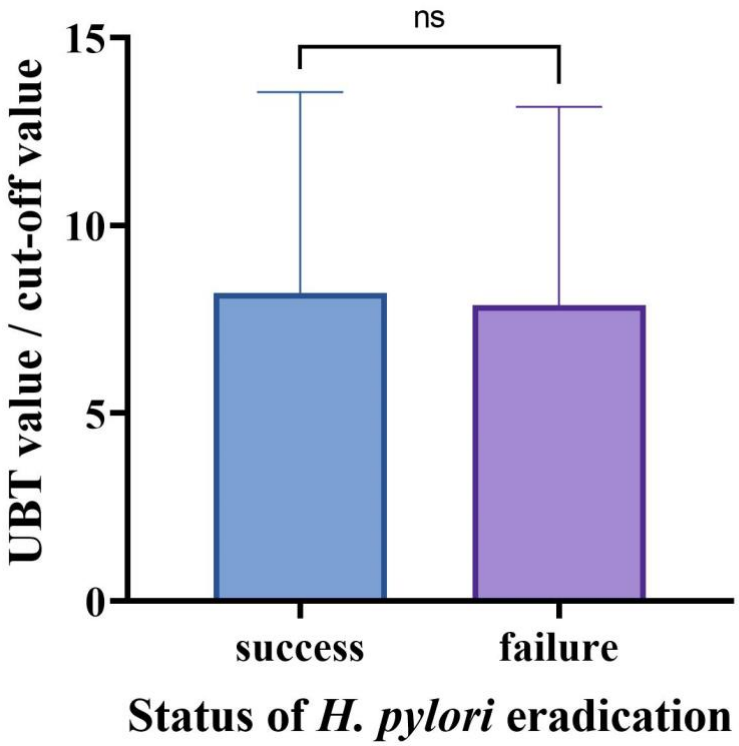

**Supplement Figure 1. Temporal trend analysis (2017–2020).**

(A) Trends in the age of patients. (B) Trends in the assessments after treatments.



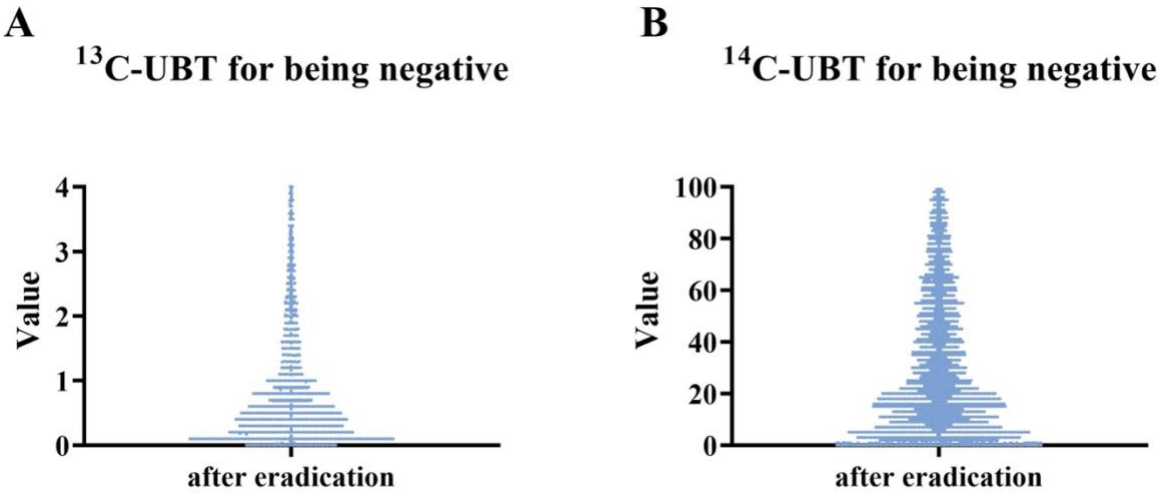

**Supplement Figure 2. Efficacy Results by treatment duration.**

(A) The eradication rate of regimens with amoxicillin and furazolidone. (B) The eradication rate of regimens with amoxicillin and clarithromycin.




**Supplement Figure 3. The association between UBT value before treatments and the outcomes.**

Data is standardized by dividing the UBT value by the cut-off value in different tests. The cut-off value of $^{13}$C-UBT was 4.0‰ (delta over baseline, DOB), and that of $^{14}$C-UBT was 100 (disintegrations per minute, DPM).




**Supplement Figure 4. Results of UBT for being negative after *H. pylori* eradication.**

(A) The scatter plot of $^{13}$C-UBT after *H. pylori* eradication. (B) The scatter plot of $^{14}$C-UBT after *H. pylori* eradication. The cut-off value of $^{13}$C-UBT was 4.0‰ (delta over baseline, DOB), and that of $^{14}$C-UBT was 100 (disintegrations per minute, DPM).

**Supplement Table 1. Effectiveness of first-line treatments per year.**

| Year | N | Success | Eradication rate (%) |
|---|---|---|---|
| 2017 | 3957 | 3317 | 83.8% |
| 2018 | 6486 | 5605 | 86.4%* |
| 2019 | 7568 | 6506 | 86.0%* |
| 2020 | 5459 | 4736 | 86.8%* |

* $P<0.05$ vs 2017.

**Supplement Table 2. Effectiveness of different first-line treatments per year.**

| Year | Regimen | N | Success | Eradication rate (%) |
|---|---|---|---|---|
| 2017 | A + F | 2785 | $2424_{a}$ | 87.0% |
| | A + C | 639 | $504_{b}$ | 78.9% |
| | F + C | 279 | $176_{c}$ | 63.1% |
| | A + L | 97 | $83_{a, b}$ | 85.6% |
| | F + L | 109 | $95_{a, b}$ | 87.2% |
| | C + L | 28 | $15_{c}$ | 53.6% |
| | Others | 20 | $20_{a, b}$ | 100.0% |
| 2018 | A + F | 4830 | $4275_{a}$ | 88.5% |
| | A + C | 863 | $750_{a}$ | 86.9% |
| | F + C | 498 | $330_{b, c}$ | 66.3% |
| | A + L | 110 | $96_{a}$ | 87.3% |
| | F + L | 122 | $107_{a}$ | 87.7% |
| | C + L | 22 | $11_{c}$ | 50.0% |
| | Others | 41 | $36_{a, b}$ | 87.8% |
| 2019 | A + F | 5009 | $4344_{a}$ | 86.7% |
| | A + C | 1853 | $1611_{a}$ | 86.9% |
| | F + C | 495 | $373_{b}$ | 75.4% |
| | A + L | 81 | $67_{a, b}$ | 82.7% |
| | F + L | 93 | $86_{a}$ | 92.5% |
| | C + L | 27 | $17_{b}$ | 63.0% |
| | Others | 10 | $8_{a, b}$ | 80.0% |
| 2020 | A + F | 4160 | $3664_{a}$ | 88.1% |
| | A + C | 827 | $712_{a}$ | 86.1% |
| | F + C | 397 | $298_{b}$ | 75.1% |
| | A + L | 21 | $18_{a, b}$ | 85.7% |
| | F + L | 34 | $30_{a, b}$ | 88.2% |
| | C + L | 18 | $13_{a, b}$ | 72.2% |
| | Others | 2 | $1_{a, b}$ | 50.0% |

Each subscript letter (a or b) denotes a subset of year categories whose column proportions do not differ significantly from each other at the .05 level. A, amoxicillin; C, clarithromycin; F, furazolidone; L, levofloxacin.

**Supplement Table 3. Recurrence after confirmation of H. pylori eradication with stricter criteria.**

| | Test | N | Standardized positivity | Recurrence rate |
|---|---|---|---|---|
| overall | overall | 1457 | 32 | 2.2% |
| | $^{13}$C-UBT | 843 | 16 | |
| | $^{14}$C-UBT | 751 | 16 | |
| A+F | overall | 1192 | 21 | 1.8% |
| | $^{13}$C-UBT | 636 | 9 | |
| | $^{14}$C-UBT | 556 | 12 | |
| A+C | overall | 242 | 5 | 2.1% |
| | $^{13}$C-UBT | 126 | 4 | |
| | $^{14}$C-UBT | 116 | 1 | |

Standardized positivity was defined by over 10‰ in $^{13}$C-UBT and over 250 in $^{14}$C-UBT. A, amoxicillin; C, clarithromycin; F, furazolidone.

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

| | Item No | Recommendation | Page No |
|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found | 1, 2 |
| **Introduction** | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 4, 5 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 5 |
| **Methods** | | | |
| Study design | 4 | Present key elements of study design early in the paper | 6 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 6 |
| Participants | 6 | (*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up | 6 |
| | | (*b*) For matched studies, give matching criteria and number of exposed and unexposed | Not applicable |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 6 |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 6 |
| Bias | 9 | Describe any efforts to address potential sources of bias | 6 |
| Study size | 10 | Explain how the study size was arrived at | 6 |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 6,7 |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding | 6,7 |
| | | (*b*) Describe any methods used to examine subgroups and interactions | 6,7 |
| | | (*c*) Explain how missing data were addressed | 6 |
| | | (*d*) If applicable, explain how loss to follow-up was addressed | 6 |
| | | (*e*) Describe any sensitivity analyses | Not applicable |
| **Results** | | | |
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | 8 |
| | | (b) Give reasons for non-participation at each stage | 8 |
| | | (c) Consider use of a flow diagram | 8 |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | 8 |
| | | (b) Indicate number of participants with missing data for each variable of interest | 8 |
| | | (c) Summarise follow-up time (eg, average and total amount) | 8 |
| Outcome data | 15* | Report numbers of outcome events or summary measures over time | 8-12 |

| | | | |
|---|---|---|---|
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included<br>(*b*) Report category boundaries when continuous variables were categorized<br>(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | 8-12<br>8-12<br>Not applicable |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | 10-12 |
| **Discussion** | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 17 |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 16, 17 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 13-16 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | 16, 17 |
| **Other information** | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 18 |

*Give information separately for exposed and unexposed groups.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

BMJ Open

# Short-term outcomes and intermediate-term follow-up of *Helicobacter pylori* infection treatment for naïve patients: A retrospective observational study

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| Keywords: | Gastroduodenal disease < GASTROENTEROLOGY, Gastrointestinal infections < GASTROENTEROLOGY, INFECTIOUS DISEASES, Diagnostic microbiology < INFECTIOUS DISEASES |
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# Short-term outcomes and intermediate-term follow-up of *Helicobacter pylori* infection treatment for naïve patients: A retrospective observational study

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## Abstract

**Objectives:** To explore the outcomes of *H. pylori* infection treatments for naïve patients in the real-world settings.

**Design:** A retrospective observational study.

**Setting:** Single tertiary level academic hospital in China.

**Participants:** We identified patients initially receiving quadruple therapy for *H. pylori* infection from 2017 to 2020 in whom eradication was confirmed (n= 23,470).

**Primary outcome:** Efficacy of different initial *H. pylori* infection treatments.

**Secondary outcome:** Results of urea breath test after *H. pylori* eradication.

**Results:** Among 23,470 patients who received initial *H. pylori* treatment, 21,285 (90.7%) were treated with amoxicillin-based regimens. The median age of the patients decreased from 2017 to 2020 (45.0 vs. 39.0, $P<0.0001$). The main treatments were therapies containing amoxicillin and furazolidone, which had an eradication rate of 87.6% (14,707 / 16,784); those containing amoxicillin and clarithromycin had an eradication rate of 85.5% (3,577 / 4,182). The date of treatment, age, antibiotic regimen and duration of treatment showed correlations with the failure of *H. pylori* eradication in a multivariable logistic regression analysis. Lastly, positive urea breath test results after eradication clustered around the cut-off value, in both the $^{13}$C-urea and $^{14}$C-urea breath tests.

**Conclusions:** The major *H. pylori* infection treatments for naïve patients were those containing amoxicillin and furazolidone, which offered the highest eradication rate. The date of treatment, age, antibiotic regimen and duration of treatment were risk factors for the failure of *H. pylori* eradication. Additionally, positive urea breath test results after eradication clustered around the cut-off value.


**Keywords:** *Helicobacter pylori*; quadruple therapy; eradication; urea breath test

**Strengths and limitations of this study**

- This observational retrospective study is based on a large clinical dataset, to avoid bias and ensure comprehensiveness.
- All data were extracted from an electronic medical record system, to ensure authenticity and relatively high completeness.
- The findings lack generalizability due to limitations of the data source; this was a single-centre study.
- Some data were inevitably missing, as the treatment protocol could not be strictly enforced.

## Introduction

*Helicobacter pylori* (*H. pylori*) is a gram-negative bacterium with prevalence varying from 24.4% to 70.1% worldwide; it accounts for over a third of global infection-attributable cancer cases [1,2]. *H. pylori* infection results in gastric diseases like chronic active gastritis and peptic ulcer disease, as well as extragastric diseases including heart diseases [3]. As *H. pylori* infection remains a major public health issue, the antibiotic resistance of *H. pylori* has increased alarmingly [4]. Fortunately, the reinfection rate remains relatively low [5]. Therefore, the effectiveness of initial *H. pylori* therapy is crucial because the rate of eradication failure increases with two or more rounds of treatment [6].

Although the prevalence of *H. pylori* infection in mainland China exhibited a slow decline of around 0.9% per year in the past decades, it is still widespread [1,7]. Successful treatment is defined as a ≥ 90% eradication rate [8]. The preferred empirical therapy for *H. pylori* infection in China [9], i.e. bismuth-containing quadruple therapy, achieved an eradication rate of 87.3% in East Asia in a recent meta-analysis [10]. Meanwhile, resistance of *H. pylori* has been increasing in recent years, and resistance to clarithromycin is considered a major cause of the failure of clarithromycin-based therapy [4,11,12]. However, the eradication rate for susceptibility-guided therapy with clarithromycin is promising, at > 95% [13]. The outcome of clarithromycin-containing therapy in real-world practice remains uncertain.

The urea breath test (UBT) is the preferred non-invasive method to detect *H. pylori* infection for initial diagnosis and assessment after treatment [14]. The principle of UBT is based on the highly active urease enzymes produced by *H. pylori*, which catalyse the reaction of a labelled urea molecule into labelled carbon dioxide that can be detected in breath samples [15]. $^{13}$C-UBT and $^{14}$C-UBT have shown similar sensitivity and specificity [16]. $^{13}$C-UBT can be used in children and pregnant women, while $^{14}$C-UBT is contraindicated for these populations because of its radioactivity [17]. It is widely acknowledged that results close to cut-off values are not reliable [9]. Setting a low cut-off value improves sensitivity while specificity remains high [18]. It is suggested that the cut-off value should also be set according to the timing of UBT, that is, before or after the eradication treatment. In most studies, UBT results in the "grey zone" were not common [19], which differs from clinical practice. Thus, the cut-off value for UBT after *H. pylori* eradication should still be set in light of new evidence.

In this study, we aimed to provide an overview of the management of *H. pylori* infection based on a large clinical dataset. The aim was to elucidate the ongoing changes in the diagnosis, treatment, and outcomes of *H. pylori* infection, to offer fresh insight into management strategies.

## Materials and Methods

### Study design and population

Patients diagnosed with *H. pylori* infection who received initial proton pump inhibitor (PPI)-bismuth-containing quadruple treatment between January 1, 2017 and December 31, 2020 were identified by searching the electronic medical records of the Second Affiliated Hospital, Zhejiang University School of Medicine (Hangzhou, China). Patients with a positive biopsy for *H. pylori* or UBT were diagnosed with *H. pylori* infection. Patients were excluded if they had previously undergone *H. pylori* eradication treatment, experienced a change in regimen during therapy, did not have their *H. pylori* infection status confirmed after eradication, or had incomplete clinical data. We retrospectively collected data from the patients' medical records, including age, sex, and treatment-related variables (date of treatment, regimen, treatment duration, and *H. pylori* eradication outcome). Data extraction was performed in September 2021. Patients' data were deidentified and two researchers checked the data independently. Supplementary File 1 shows the detailed study protocol.

### Patient and public involvement

Patients and/or the public were not involved in the design, conduct, reporting or dissemination plans for this research.

### Exposure

Gastroenterologists inquired about treatment-naïve patients' history of antibiotic exposure before prescribing treatment, and determined the treatment based on their clinical experience. The prescription was recorded in the electronic medical record system. We focused on bismuth-containing quadruple therapies (PPI + bismuth + two antibiotics), which were recommended as the main empirical therapy for *H. pylori* eradication in China [9]. Different first-line treatments were classified into seven categories, and PPIs into six categories, according to the fifth Chinese National Consensus Report on the management of *Helicobacter pylori* infection (Supplementary File 2).

### Follow-up and Outcomes

Follow-up was performed through outpatient clinical visits. Patients were asked to visit the outpatient clinics over a period of at least 4 weeks after completion of *H. pylori* therapy. Eradication of

*H. pylori* infection was confirmed by $^{13}$C-UBT or $^{14}$C-UBT at least 4 weeks after therapy. $^{14}$C-UBT was contraindicated in children and pregnant women because of its radioactivity. Patients were informed about the similar sensitivity and specificity of $^{13}$C-UBT or $^{14}$C-UBT and the different costs and contraindications due to radioactivity. The patients chose the treatments themselves. The cut-off value for $^{13}$C-UBT was 4.0‰ (delta over baseline, DOB), and that of $^{14}$C-UBT was 100 (disintegrations per minute, DPM). Patients were not permitted to take any PPIs 2 weeks prior to the UBT, or any antibiotics 4 weeks before the UBT.

**Univariate and multivariable logistic analyses**

A binary logistic regression analysis was performed to examine the relationship between the failure of *H. pylori* eradication and various factors. Patients who did not receive regimens with amoxicillin plus furazolidone, amoxicillin plus clarithromycin, or furazolidone plus clarithromycin were not included in the analyses. Patients who received 12-day treatment were also excluded from the analyses. Covariates were included in the multivariable model when their *P* values were < 0.1 in univariate analysis, when adding the covariate to the model changed the OR by > 10%, or on the basis of previous findings. After verifying the stability of the results among different models, we derived the final model using the forward stepwise method (likelihood ratio; criterion for model inclusion and removal = 0.05 and 0.10, respectively).

**Statistical analyses**

Non-normally distributed continuous variables are presented as median (interquartile range) and categorical variables as absolute frequencies (proportions). The primary outcome was the *H. pylori* infection eradication rate. Continuous variables were compared using the nonparametric Kruskal-Wallis test. Categorical variables were compared using the chi-square test. Statistical significance was defined as $P < 0.05$. Statistical analyses were performed using SPSS (version 26.0; SPSS Inc., Chicago, IL, USA) and GraphPad PRISM software (ver. 9.0; GraphPad Software, Inc., San Diego, CA, USA).

## Results

From January 2017 to December 2020, 25,796 naïve patients diagnosed with *H. pylori* infection received PPI-bismuth-containing quadruple therapy and took a UBT for at least 4 weeks after the treatment. Among those patients, 23,470 (91%) were included in the analysis (Figure 1). Most of the patients (90.7%, 21,285 / 23,470) were treated with amoxicillin-based regimens.

### Baseline characteristics

The patients' baseline demographic and clinical characteristics are presented in Table 1.

**Table 1. Baseline characteristics**

| Characteristics | |
|---|---|
| Overall cases | 23470 |
| Age, median (IQR) | 40 (30–54) |
| Sex, N (%) | |
| Male | 11008 (46.9) |
| Female | 12462 (53.1) |
| Date of treatment, N (%) | |
| 2017 | 3957 (16.9) |
| 2018 | 6486 (27.6) |
| 2019 | 7568 (32.2) |
| 2020 | 5459 (23.3) |
| Season, N (%) | |
| Spring | 4473 (19.1) |
| Summer | 5942 (25.3) |
| Autumn | 6322 (26.9) |
| Winter | 6733 (28.7) |
| Antibiotic regimens, N (%) | |
| Amoxicillin + furazolidone | 16784 (71.5) |
| Amoxicillin + clarithromycin | 4182 (17.8) |
| Amoxicillin + levofloxacin | 309 (1.3) |
| Furazolidone + clarithromycin | 1669 (7.1) |
| Furazolidone + levofloxacin | 358 (1.5) |
| Clarithromycin + levofloxacin | 95 (0.4) |
| Others | 73(0.3) |
| Duration, N (%) | |
| 10 | 5641(24.0) |
| 12 | 1060(4.5) |
| 14 | 16769(71.4) |
| Proton pump inhibitor, N (%) | |
| Rabeprazole10mg | 9654(41.1) |
| Rabeprazole20mg | 21(0.1) |

| | |
|---|---|
| Pantoprazole | 5815(24.8) |
| Esomeprazole | 4811(20.5) |
| Omeprazole | 2236(9.5) |
| Lansoprazole | 933(4.0) |

**Time-trend analysis**

Supplementary Figure 1A depicts the age distribution among people who received *H. pylori* eradication treatment from 2017 to 2020. Bimodal distributions existed for all groups, with an increase in the number of young patients occurring over time. The median age was 45.0 (33.0–54.0) in 2017, 40.0 (31.0–54.0) in 2018, 39.0 (30.0–53.0) in 2019 and 39.0 (30.0–54.0) in 2020. Supplementary Figure 1B shows relative proportions of different UBTs used; an increase in the use of $^{14}$C-UBT over time can be seen, from 42.2% in 2017 to 59.6% in 2020 ($P < 0.05$).

**Efficacy results**

The overall *H. pylori* infection eradication rate rose considerably from 83.8% in 2017 to 86.8% in 2020 (Supplementary Table 1). Figure 2A shows that amoxicillin-based therapies achieved a higher cure rate than amoxicillin-free therapies every year during the time frame (85.5% vs. 70.1%, $P < 0.001$ in 2017; 88.3% vs. 70.8%, $P < 0.001$ in 2018; 86.7% vs. 77.4%, $P < 0.001$ in 2019 and 87.7% vs. 75.8%, $P < 0.001$ in 2020). Figure 2B depicts the eradication rate of three dominant regimens by date of treatment. The eradication rate of therapies containing amoxicillin and furazolidone was higher than that of therapies containing amoxicillin and clarithromycin in 2017 (87.0% vs. 78.9%, $P < 0.001$). However, there was no significant difference between these two therapies in later years (88.5% vs. 86.9%, $P = 0.178$ in 2018; 86.7% vs. 86.9%, $P = 0.814$ in 2019 and 88.1% vs. 86.1%, $P = 0.112$ in 2020). During the 4-year period, therapies containing furazolidone and clarithromycin had the lowest cure rate (63.1% in 2017, 66.3% in 2018, 75.4% in 2019, and 75.1% in 2020). The high eradication rates of the other therapies might be inconsistent with real-world practice due to the small sample size (Supplementary Table 2).

The eradication rates were 89.5%, 87.2%, 85.6%, 83.3%, and 80.4% in patients aged ≤ 30, 31–40, 41–50, 51–60, and > 60 years, respectively ($P < 0.001$, Figure 2C), indicating a higher eradication rate among younger patients. The same age trend was also observed for therapies containing amoxicillin and furazolidone, and for those containing amoxicillin and clarithromycin (Figure 2D). In patients aged ≤

30 and 51–60 years, therapies containing amoxicillin and furazolidone had better outcomes than therapies containing amoxicillin and clarithromycin (90.8% vs. 87.8%, $P = 0.002$ and 85.2% vs. 81.8%, $P = 0.020$). Patients aged > 60 years old had the lowest cure rate with both kinds of therapies (82.8% vs. 78.9%, $P = 0.052$).

We also analysed how the treatment duration and UBT result before treatment impacted the *H. pylori* eradication (Supplementary Figure 2). Generally, there is little significant difference between 10- and 14-day therapies with amoxicillin and furazolidone. For therapies including amoxicillin and clarithromycin, 14-day treatment provided a better result than 10-day treatment in 2019 (88.8% vs. 82.8%, $P = 0.002$), but no significant difference was observed in 2020 (84.6% vs 88.4%, $P = 0.233$). The UBT value before treatment was approximately the same regardless of whether the eradication succeeded ($P > 0.05$, Supplementary Figure 3).

**Multivariable logistic regression analysis of the failure of *H. pylori* eradication**

We used a logistic regression model to identify factors predicting the failure of *H. pylori* eradication (Table 2). We derived the final model using the forward stepwise method (likelihood ratio; criterion for model inclusion and removal = 0.05 and 0.10, respectively; Hosmer and Lemeshow test statistic, $P = 0.652$). The multivariable analysis showed that age, date of treatment, antibiotic regimen, and treatment duration were associated with the poor outcomes, while sex, season, and PPI use were not.

**Table 2. Univariate and multivariable analyses of risk factors for *H. pylori* eradication failure.**

| Characteristics | N | Univariate analysis | | Multivariable analysis | |
|---|---|---|---|---|---|
| | | OR (95%CI) | *P*-value | OR (95%CI) | *P*-value |
| Age | | | | | |
| ≤ 30 | 5400 | 1.00 | – | 1.00 | – |
| 30 – 40 | 5634 | 1.25(1.12–1.41) | < 0.001 | 1.25(1.11–1.41) | < 0.001 |
| 40 – 50 | 3774 | 1.43(1.26–1.62) | < 0.001 | 1.43(1.26–1.62) | < 0.001 |
| 50 – 60 | 4310 | 1.68(1.49–1.89) | < 0.001 | 1.70(1.51–1.91) | < 0.001 |
| > 60 | 2586 | 2.04(1.79–2.32) | < 0.001 | 2.01(1.76–2.29) | < 0.001 |
| Sex | | | | | |
| Male | 10257 | 1.00 | – | | |
| Female | 11447 | 0.98(0.91–1.06) | 0.646 | | |
| Date of treatment | | | | | |
| 2017 | 3695 | 1.00 | – | 1.00 | – |
| 2018 | 6123 | 0.82(0.73–0.91) | < 0.001 | 0.85(0.75–0.95) | 0.005 |
| 2019 | 6740 | 0.86(0.77–0.96) | 0.007 | 0.90(0.80–1.01) | 0.071 |
| 2020 | 5146 | 0.80(0.71–0.90) | < 0.001 | 0.86(0.76–0.97) | 0.017 |
| Season | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| Spring | 4008 | 1.00 | – | | |
| Summer | 5489 | 0.86(0.77–0.96) | 0.009 | | |
| Autumn | 5828 | 0.88(0.78–0.98) | 0.021 | | |
| Winter | 6379 | 0.88(0.79–0.98) | 0.024 | | |
| Antibiotic regimens | | | | | |
| Amoxicillin + furazolidone | 16230 | 1.00 | – | 1.00 | – |
| Amoxicillin + clarithromycin | 3885 | 1.19(1.08–1.32) | 0.001 | 1.21(1.09–1.34) | < 0.001 |
| Furazolidone + clarithromycin | 1589 | 2.99(2.66–3.36) | < 0.001 | 2.97(2.64–3.34) | < 0.001 |
| Duration | | | | | |
| 10 | 5348 | 1.00 | – | 1.00 | – |
| 14 | 16356 | 0.81(0.75–0.89) | < 0.001 | 0.89(0.82–0.97) | 0.011 |
| Proton pump inhibitor | | | | | |
| Rabeprazole10mg | 8826 | 1.00 | – | | |
| Rabeprazole20mg | 21 | 1.05(0.31–3.55) | 0.944 | | |
| Pantoprazole | 5455 | 1.11(1.01–1.22) | 0.029 | | |
| Esomeprazole | 4530 | 1.11(1.00–1.23) | 0.049 | | |
| Omeprazole | 2010 | 0.83(0.72–0.96) | 0.014 | | |
| Lansoprazole | 862 | 1.02(0.84–1.25) | 0.818 | | |

**Specificity of urea breath test after *H. pylori* eradication**

Figure 3 shows the data of positive $^{13}$C-UBT and $^{14}$C-UBT for naïve patients before and after eradication treatment. The median UBT value for patients positive before eradication treatment was much higher than that after treatment (23.40 (14.30–34.90) vs. 12.30 (6.50–24.60) for $^{13}$C-UBT, $P <$ 0.0001; 1118.0 (636.0–1702.0) vs. 303.0 (146.0–930.0) for $^{14}$C-UBT, $P < 0.0001$). The results for positive UBT patients after eradication clustered around the cut-off value, but this is not seen for those with negative results (Supplementary Figure 4).

**Recurrence after confirmation of *H. pylori* eradication with stricter criteria**

Successful *H. pylori* eradication with stricter criteria was determined based on the UBT performed at least 8 weeks after the end of the initial *H. pylori* eradication treatment[5]. Recurrence was determined based on a UBT result > 2.5 times higher than the cut-off value after successful eradication. Among 10,056 patients for whom *H. pylori* eradication was successful, 1,617 retook the UBT (23 had qualitative but not quantitative data). The $^{13}$C-UBT result for 16 of 843 individuals was > 10‰, and the $^{13}$C-UBT results for 16 of 751 individuals was > 250 (Supplementary Table 3). The overall recurrence rate was 2.2%. For patients who received the amoxicillin-furazolidone and amoxicillin-clarithromycin regimens, the recurrence rates were 1.8% and 2.1%, respectively; there was no significant difference between these rates.

## Discussion

In this large-scale retrospective study, we present the outcomes and follow-up data of initial *H. pylori* treatments performed over a 4-year period in patients seen at a single centre in East China.

The recommendation that *H. pylori*-positive individuals receive early eradication treatment, to benefit both themselves and society, led to a shift in practice [20]. The indication for *H. pylori* eradication were also expanded in China, where eradication is recommended for confirmed *H. pylori* infection cases [9]. We observed that the age of patients decreased over time. There were two main age clusters, i.e. young and middle-aged. The risk profiles of these two groups differed, such that they were treated via two different strategies.

From 2017 to 2020, *H. pylori* treatments included 21,285 amoxicillin-based regimens and 2,185 amoxicillin-free regimens. The eradication rate of amoxicillin-free treatments was much lower than that of amoxicillin-based treatments. Amoxicillin is considered as a major component of *H. pylori* treatment in case of low resistance [4]. Doctors should carefully investigate documented patient allergies to penicillin. Previous reports indicate that most patients who claim to be allergic to penicillin ultimately have a negative skin test [21-23]. Moreover, *H. pylori* might correlate with the occurrence and persistence of chronic spontaneous urticaria [24], which could result in false positive skin tests. In addition, some patients mistook adverse reactions, such as nausea, for allergy. Detailed information should be recorded to help us identify the truly allergic patients. Furthermore, only one fatal case of anaphylaxis in the UK between 1972 and 2007 was potentially associated with oral amoxicillin [25]. De-labelling penicillin allergy is currently of great concern, and direct challenge might be a safe and effective approach [26]. Based on the existing evidence, physicians should have more confidence in the safety of oral amoxicillin.

Amoxicillin treatment schemes involving furazolidone or clarithromycin are the most widely used, and are both generally prescribed as 14-day regimens. We could discern an effect of an updated guideline not recommending levofloxacin for initial treatment (i.e. few prescriptions thereof) [9]. *H. pylori* remains highly sensitive to amoxicillin, furazolidone, and tetracycline in China, especially East China[12]. Antibiotic regimens with amoxicillin and furazolidone dominated in the past few years, as tetracycline was not available in our hospital pharmacy. However, furazolidone is not used in some countries despite

its low resistance. The Federal Drug Agency warns that furazolidone may reduce fertility or injure unborn children [27]. Nevertheless, the International Agency for Research on Cancer classified furazolidone into group 3, i.e. not carcinogenic in humans [28]. The Shire company stopped marketing furazolidone, and eventually withdrew it because of poor sales [29]. According to a meta-analysis, a 14-day furazolidone-containing regimen with a low daily dose of 200 mg was well-tolerated and should be uses as first-line treatment [30]. No serious adverse events were reported among the cases in our study. Furazolidone-containing therapies with a high eradication rate should be re-evaluated in other countries.

Clarithromycin resistance has increased in the Asia-Pacific region in the past few decades, presumably due to the increasing consumption of macrolides [31-33]. Clarithromycin-containing regimens are not recommended in areas where clarithromycin resistance exceeds 20% [32]. However, the effectiveness of regimens with amoxicillin and clarithromycin was similar to that of regimens with amoxicillin and furazolidone from 2018 to 2020. According to updated guidelines, the gastroenterologists in our hospital were instructed to inquire about the patients' history of antibiotic exposure before prescription, which might explain the contradictory results. This suggests that we should further investigate regimens involving clarithromycin for *H. pylori* eradication, and focus on patient populations for whom they may be effective. In a population with high resistance to clarithromycin, metronidazole, and levofloxacin, susceptibility-guided therapies and a highly effective empiric regimen both achieved eradication levels > 95% [13]. The latter treatment should be preferred, considering its simplicity. Given the controversy over the empiric regimen of choice in our region, further prospective studies are warranted for this scenario.

Factors associated with eradication failure in this study included the date of treatment and duration, patient age, and antibiotic regimen. Patients who received therapies during the period 2018–2020 were less likely to experience eradication failure than those in 2017, when the new expert consensus report was published. There might be a relationship between eradication outcomes and clinicians' knowledge of clinical practice guidelines. Moreover, failure of *H. pylori* eradication was more likely in older patients. However, the "test and treat" strategy is not recommended for young children; it is considered unnecessary until middle-school age Japan, and in those aged ≤ 14 years in China [9,14,34]. However,

screening among high-school students and undergraduates might be an important measure to improve the eradication rate, reduce the risk of gastric cancer, and prevent transmission, although a lower eradication rate has also been reported in younger patients, especially those with gastric ulcers [35]. Symptoms and endoscopic and pathological findings suggest varying pathologic mechanisms of *H. pylori* infection. Thus, these factors should be assessed in future studies to determine the relationship between age and eradication outcomes. Consistent with the recommendation of the Maastricht V/Florence Consensus Report that the treatment duration of bismuth quadruple therapy be extended to 14 days [17], our work showed a slight positive correlation between the treatment duration and outcome. However, the difference between the two major therapies was not significant; this should be further investigated.

In agreement with a prospective study, we observed no association between the UBT value before treatment and *H. pylori* eradication status [36]. Also, patient outcomes were not significantly different according to the PPI used. However, a meta-analysis reported higher cure rates with new-generation PPIs (esomeprazole and rabeprazole) than first-generation PPIs (omeprazole, lansoprazole and pantoprazole), especially in CYP2C19 extensive metabolizers [37]. Other factors such as adherence to treatment, cigarette smoking, and genetic factors, also played a role [38,39]. These factors should be further explored in future investigations.

UBT is recommended after *H. pylori* eradication, with the monoclonal feacal antigen test serving as an alternative [9]. However, the monoclonal feacal antigen test was not available in our hospital until November 2021. After radioactive drugs containing 1 μCi of carbon-14 urea were approved [40], the use of $^{14}$C-UBT increased over time, albeit less than predicted considering its economic benefits. Unexpectedly, after eradication treatment, UBT results close to the cut-off value were not uncommon in our cohort. Long-term follow-up of $^{13}$C-UBT results after *H. pylori* eradication suggested that a lower cut-off value may improve diagnostic accuracy, based on the changes seen in the gastric density of microorganisms [18]. Negative UBT results were not clustered around the cut-off, in contrast to the positive ones. This might lead to misclassification of the eradication failure and underestimation of the eradication rate. However, the stool antigen test is even less accurate for patients after *H. pylori*

eradication, and thus has lower positive predictive value than the UBT [41,42]. Further studies are needed to address this important but overlooked issue.

This study had several limitations. Firstly, retrospective studies do not permit definite conclusions to be drawn, and some bias is inevitable. Furthermore, the follow-up schedule was not as strict as would have been the case in a prospective study. Secondly, patient information was incomplete. Factors such as prior antibiotic exposure, resistance to antibiotics, treatment compliance/adherence, smoking history, the *H. pylori* infection status of family members, socioeconomic status, and hygiene status were not analysed. Thirdly, although there are other first-line treatment regimens for *H. pylori* infection [10], we only focused on therapies containing PPI and bismuth, especially amoxicillin-based therapies including furazolidone or clarithromycin. Vonoprazan, as a potent new acid inhibitor, has not yet been approved for *H. pylori* infection in China [43]. Vonoprazan-based therapies achieved eradication rates > 90%, indicating promise for *H. pylori* infection treatment.

With improved understanding and greater public attention, the treatment options for *H. pylori* infection, especially for young people, might increase. Amoxicillin-free regimens accounted for 9.3% of the treatments in our cohort. Doctors should be aware of the importance of amoxicillin and correct concept of penicillin allergy. Regimens involving amoxicillin and furazolidone were the most widely used among our cohort, presumably due to the generally acknowledged increasing resistance of *H. pylori* to clarithromycin and levofloxacin. However, the observed effectiveness of quadruple therapy containing amoxicillin-clarithromycin contradicts this. Notably, $^{13}$C-UBT and $^{14}$C-UBT results after *H. pylori* eradication clustered around the cut-off value, which suggests the need to review current mainstream diagnostic methods. Further studies to confirm the effectiveness of different regimens, and the specificity of UBT for *H. pylori* diagnosis, are needed.

## STATEMENTS & DECLARATIONS

**Contributions:** Conception and design: Du Q, Ye J and Wang Y. Acquisition of data: Wang Y, Xiang Y, Liao O, Wu Y and Li Y. Analysis or interpretation of the data: Wang Y, Xiang Y and Ye J. Drafting of the manuscript: Wang Y and Ye J. Revision of the manuscript: Ye J, Du Q and Li Y. Study guarantor and supervision: Du Q and Ye J.

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**Competing interests:** The authors declare that they have no competing interests.

**Patient consent:** All data were collected with de-identified personal information to ensure that individuals maintained their anonymity. This study was exempted from obtaining individual informed consent as the study was based on routine de-identified data.

**Ethics approval:** The study was approved by the Ethics Committee of the Second Affiliated Hospital, Zhejiang University School of Medicine (registration no. 2021-0716).

**Data availability statement:** Data are available from the corresponding author upon reasonable request.

## References


1. Hooi JKY, Lai WY, Ng WK, et al. Global Prevalence of Helicobacter pylori Infection: Systematic Review and Meta-Analysis. *Gastroenterology*. 2017;153(2):420-429.
2. de Martel C, Georges D, Bray F, Ferlay J, Clifford GM. Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis. *The Lancet. Global health.* 2020;8(2):e180-e190.
3. Robinson K, Atherton JC. The Spectrum of -Mediated Diseases. *Annual review of pathology.* 2021;16:123-144.
4. Savoldi A, Carrara E, Graham DY, Conti M, Tacconelli E. Prevalence of Antibiotic Resistance in Helicobacter pylori: A Systematic Review and Meta-analysis in World Health Organization Regions. *Gastroenterology.* Nov 2018;155(5):1372-1382 e1317.
5. Xie Y, Song C, Cheng H, et al. Long-term follow-up of reinfection and its risk factors after initial eradication: a large-scale multicentre, prospective open cohort, observational study. *Emerging microbes & infections.* 2020;9(1):548-557.
6. Shah SC, Iyer PG, Moss SF. AGA Clinical Practice Update on the Management of Refractory Helicobacter pylori Infection: Expert Review. *Gastroenterology.* Apr 2021;160(5):1831-1841.
7. Li M, Sun Y, Yang J, et al. Time trends and other sources of variation in Helicobacter pylori infection in mainland China: A systematic review and meta-analysis. *Helicobacter.* Oct 2020;25(5):e12729.
8. Graham DY, Lee Y-C, Wu M-S. Rational Helicobacter pylori therapy: evidence-based medicine rather than medicine-based evidence. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association.* 2014;12(2).
9. Liu WZ, Xie Y, Lu H, et al. Fifth Chinese National Consensus Report on the management of Helicobacter pylori infection. *Helicobacter.* 2018;23(2):e12475.
10. Rokkas T, Gisbert JP, Malfertheiner P, et al. Comparative Effectiveness of Multiple Different First-Line Treatment Regimens for Helicobacter pylori Infection: A Network Meta-analysis. *Gastroenterology.* 2021;161(2).
11. Thung I, Aramin H, Vavinskaya V, et al. Review article: the global emergence of Helicobacter pylori antibiotic resistance. *Alimentary pharmacology & therapeutics.* 2016;43(4):514-533.
12. Zhong Z, Zhang Z, Wang J, et al. A retrospective study of the antibiotic-resistant phenotypes and genotypes of strains in China. *American journal of cancer research.* 2021;11(10):5027-5037.
13. Chen Q, Long X, Ji Y, et al. Randomised controlled trial: susceptibility-guided therapy versus empiric bismuth quadruple therapy for first-line Helicobacter pylori treatment. *Alimentary pharmacology & therapeutics.* 2019;49(11):1385-1394.
14. Kato M, Ota H, Okuda M, et al. Guidelines for the management of Helicobacter pylori infection in Japan: 2016 Revised Edition. *Helicobacter.* 2019;24(4):e12597.
15. Keller J, Hammer HF, Afolabi PR, et al. European guideline on indications, performance and clinical impact of (13) C-breath tests in adult and pediatric patients: An EAGEN, ESNM, and ESPGHAN consensus, supported by EPC. *United European Gastroenterol J.* Jun 2021;9(5):598-625.

16. Ferwana M, Abdulmajeed I, Alhajiahmed A, et al. Accuracy of urea breath test in Helicobacter pylori infection: meta-analysis. *World J Gastroenterol.* Jan 28 2015;21(4):1305-1314.
17. Malfertheiner P, Megraud F, O'Morain CA, et al. Management of Helicobacter pylori infection-the Maastricht V/Florence Consensus Report. *Gut.* 2017;66(1).
18. Gisbert JP, Olivares D, Jimenez I, Pajares JM. Long-term follow-up of 13C-urea breath test results after Helicobacter pylori eradication: frequency and significance of borderline delta13CO2 values. *Alimentary pharmacology & therapeutics.* 2006;23(2):275-280.
19. Kato C, Sugiyama T, Sato K, et al. Appropriate cut-off value of 13C-urea breath test after eradication of Helicobacter pylori infection in Japan. *Journal of gastroenterology and hepatology.* 2003;18(12):1379-1383.
20. Sugano K, Tack J, Kuipers EJ, et al. Kyoto global consensus report on Helicobacter pylori gastritis. *Gut.* 2015;64(9):1353-1367.
21. Chey WD, Leontiadis GI, Howden CW, Moss SF. ACG Clinical Guideline: Treatment of Helicobacter pylori Infection. *The American journal of gastroenterology.* 2017;112(2):212-239.
22. Shenoy ES, Macy E, Rowe T, Blumenthal KG. Evaluation and Management of Penicillin Allergy: A Review. *JAMA.* 2019;321(2):188-199.
23. Stone CA, Trubiano J, Coleman DT, Rukasin CRF, Phillips EJ. The challenge of de-labeling penicillin allergy. *Allergy.* 2020;75(2):273-288.
24. Kim HJ, Kim Y-J, Lee HJ, et al. Systematic review and meta-analysis: Effect of Helicobacter pylori eradication on chronic spontaneous urticaria. *Helicobacter.* 2019;24(6):e12661.
25. Lee P, Shanson D. Results of a UK survey of fatal anaphylaxis after oral amoxicillin. *The Journal of antimicrobial chemotherapy.* 2007;60(5):1172-1173.
26. Mustafa SS, Conn K, Ramsey A. Comparing Direct Challenge to Penicillin Skin Testing for the Outpatient Evaluation of Penicillin Allergy: A Randomized Controlled Trial. *The journal of allergy and clinical immunology. In practice.* 2019;7(7):2163-2170.
27. https://dailymed.nlm.nih.gov/dailymed/fda/fdaDrugXsl.cfm?setid=056f4352-baaa-4f47-9fb3-fc2bdde6b26e.
28. https://monographs.iarc.who.int/list-of-classifications.
29. Graham DY, Lu H. Furazolidone in Helicobacter pylori therapy: misunderstood and often unfairly maligned drug told in a story of French bread. *Saudi journal of gastroenterology : official journal of the Saudi Gastroenterology Association.* 2012;18(1):1-2.
30. Ji C-R, Liu J, Li Y-Y, et al. Safety of furazolidone-containing regimen in infection: a systematic review and meta-analysis. *BMJ open.* 2020;10(10):e037375.
31. Megraud F, Bruyndonckx R, Coenen S, et al. resistance to antibiotics in Europe in 2018 and its relationship to antibiotic consumption in the community. *Gut.* 2021;70(10):1815-1822.
32. Kuo Y-T, Liou J-M, El-Omar EM, et al. Primary antibiotic resistance in Helicobacter pylori in the Asia-Pacific region: a systematic review and meta-analysis. *The lancet. Gastroenterology & hepatology.* 2017;2(10):707-715.
33. Kocsmár É, Buzás GM, Szirtes I, et al. Primary and secondary clarithromycin resistance in Helicobacter pylori and mathematical modeling of the role of macrolides. *Nature communications.* 2021;12(1):2255.

**34.** Jones NL, Koletzko S, Goodman K, et al. Joint ESPGHAN/NASPGHAN Guidelines for the Management of Helicobacter pylori in Children and Adolescents (Update 2016). *Journal of pediatric gastroenterology and nutrition.* 2017;64(6).

**35.** Tang Y, Tang G, Pan L, Zhu H, Zhou S, Wei Z. Clinical factors associated with initial Helicobacter pylori eradication therapy: a retrospective study in China. *Sci Rep.* Sep 21 2020;10(1):15403.

**36.** Gisbert JP, Olivares D, Jimenez I, Pajares JM. Is there any correlation between 13C-urea breath test values and response to first-line and rescue Helicobacter pylori eradication therapies? *Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver.* 2006;38(4):254-259.

**37.** McNicholl AG, Linares PM, Nyssen OP, Calvet X, Gisbert JP. Meta-analysis: esomeprazole or rabeprazole vs. first-generation pump inhibitors in the treatment of Helicobacter pylori infection. *Alimentary pharmacology & therapeutics.* 2012;36(5):414-425.

**38.** Yu J, Yang P, Qin X, Li C, Lv Y, Wang X. Impact of smoking on the eradication of Helicobacter pylori. *Helicobacter.* 2021:e12860.

**39.** Graham DY, Lew GM, Malaty HM, et al. Factors influencing the eradication of Helicobacter pylori with triple therapy. *Gastroenterology.* 1992;102(2):493-496.

**40.** https://www.nrc.gov/reading-rm/doc-collections/cfr/part030/part030-0021.html.

**41.** Bilardi C, Biagini R, Dulbecco P, et al. Stool antigen assay (HpSA) is less reliable than urea breath test for post-treatment diagnosis of Helicobacter pylori infection. *Alimentary pharmacology & therapeutics.* 2002;16(10):1733-1738.

**42.** Perri F, Manes G, Neri M, Vaira D, Nardone G. Helicobacter pylori antigen stool test and 13C-urea breath test in patients after eradication treatments. *The American journal of gastroenterology.* 2002;97(11):2756-2762.

**43.** Kagami T, Sahara S, Ichikawa H, et al. Potent acid inhibition by vonoprazan in comparison with esomeprazole, with reference to CYP2C19 genotype. *Alimentary pharmacology & therapeutics.* 2016;43(10):1048-1059.

**Figure Legends**

**Figure 1. Study flow chart.**

**Figure 2. Efficacy Results**

(A) The eradication rate of amoxicillin-based regimens and amoxicillin-free regimens. (B) The eradication rate of three dominant therapies by date of treatment. (C) The eradication rate by age. (D) The eradication rate of two dominant therapies by age. A, amoxicillin; C, clarithromycin; F, furazolidone.

**Figure 3. Results of UBT for being positive before and after *H. pylori* eradication.**

(A) The scatter plot of positive $^{13}$C-UBT results before and after *H. pylori* eradication. (B) The scatter plot of positive $^{14}$C-UBT results before and after *H. pylori* eradication. The cut-off value of $^{13}$C-UBT was 4.0‰ (delta over baseline, DOB), and that of $^{14}$C-UBT was 100 (disintegrations per minute, DPM).

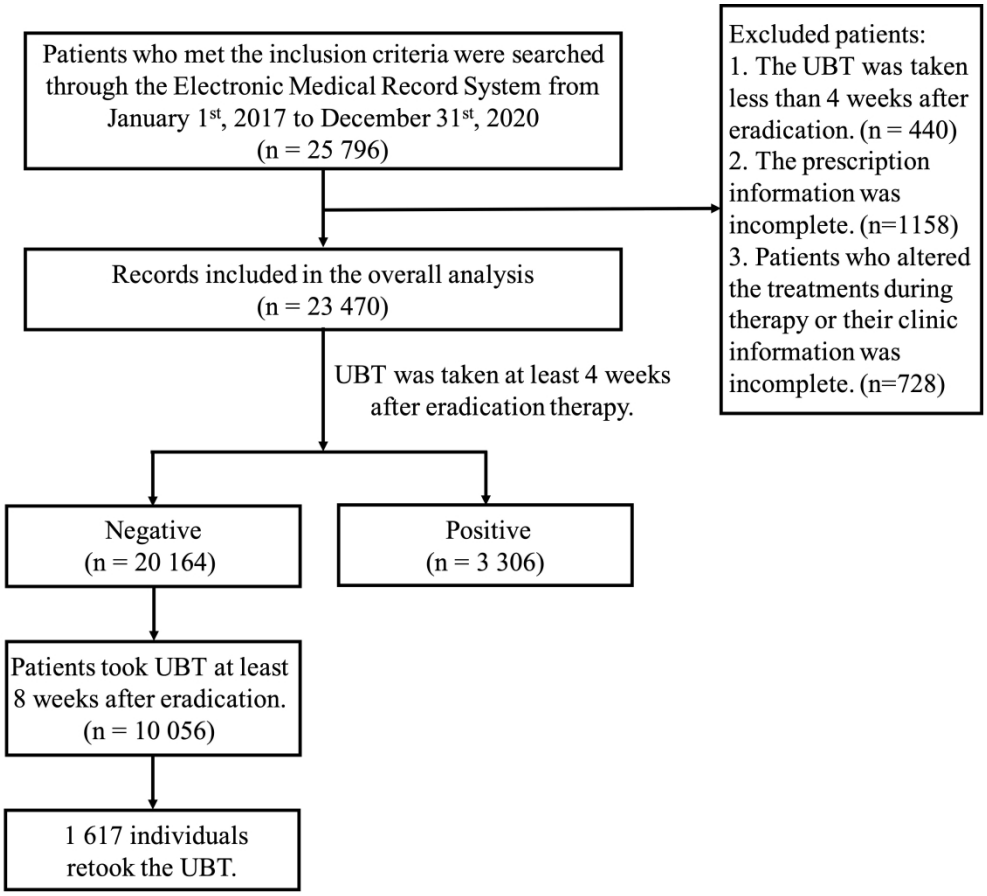



Figure 1. Study flow chart.

1334x1193mm (72 x 72 DPI)

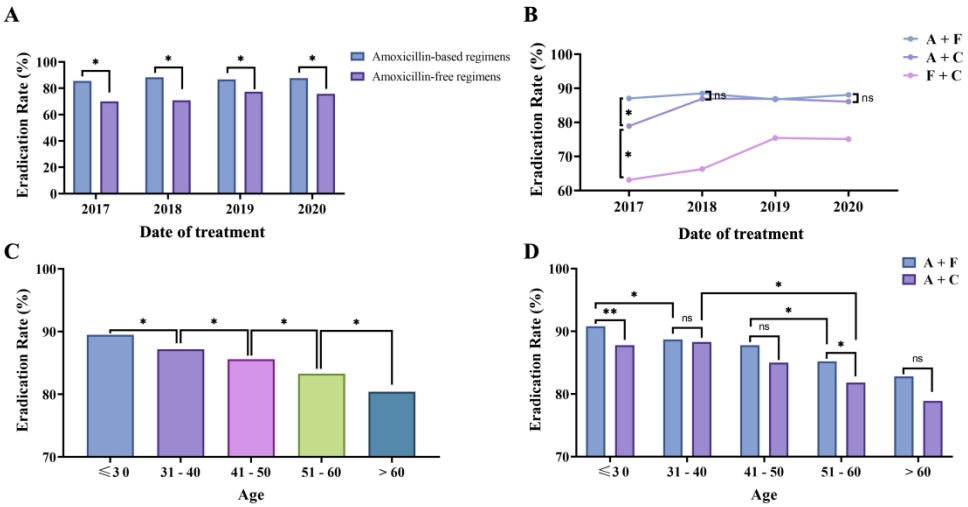



Figure 2. Efficacy Results
(A) The eradication rate of amoxicillin-based regimens and amoxicillin-free regimens. (B) The eradication rate of three dominant therapies by date of treatment. (C) The eradication rate by age. (D) The eradication rate of two dominant therapies by age. A, amoxicillin; C, clarithromycin; F, furazolidone.

1590x824mm (72 x 72 DPI)

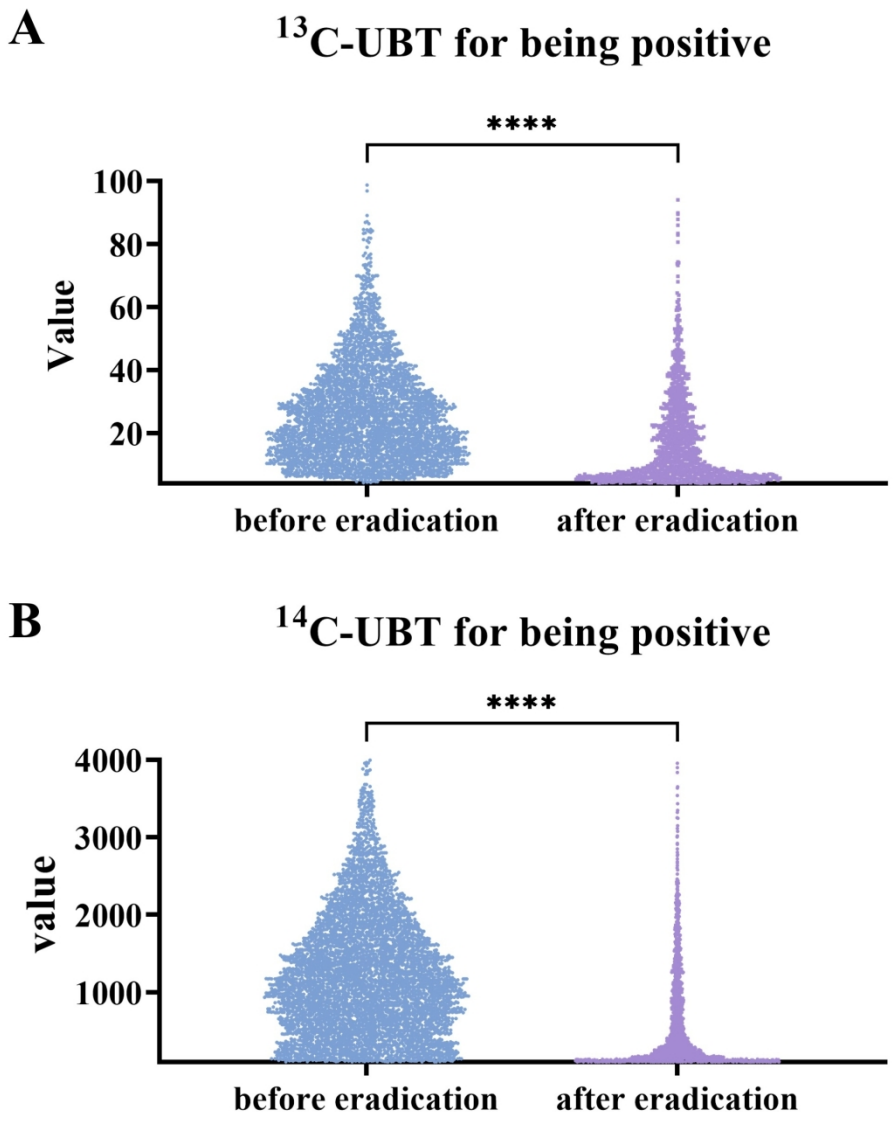



Figure 3. Results of UBT for being positive before and after H. pylori eradication.(A) The scatter plot of positive $^{13}$C-UBT results before and after H. pylori eradication. (B) The scatter plot of positive $^{14}$C-UBT results before and after H. pylori eradication. The cut-off value of 13C-UBT was 4.0‰ (delta over baseline, DOB), and that of 14C-UBT was 100 (disintegrations per minute, DPM).

165x191mm (330 x 330 DPI)

# ONLINE SUPPLEMENTARY MATERIAL

## Supplementary File 1. The original protocol for the study

Data were searched through Electronic Medical Record System of the Second Affiliated Hospital, Zhejiang University School of Medicine (Hangzhou, China). Data extraction was performed in September 2021 by IT department.

Inclusion criteria:

1. time frame (from January 1st 2017 to December 31st 2020)
2. diagnosed with *H. pylori* infection
3. received initial PPI-bismuth-containing quadruple treatments
4. with the results of urea breath test at least 4 weeks after eradication therapy

Exclusion criteria:

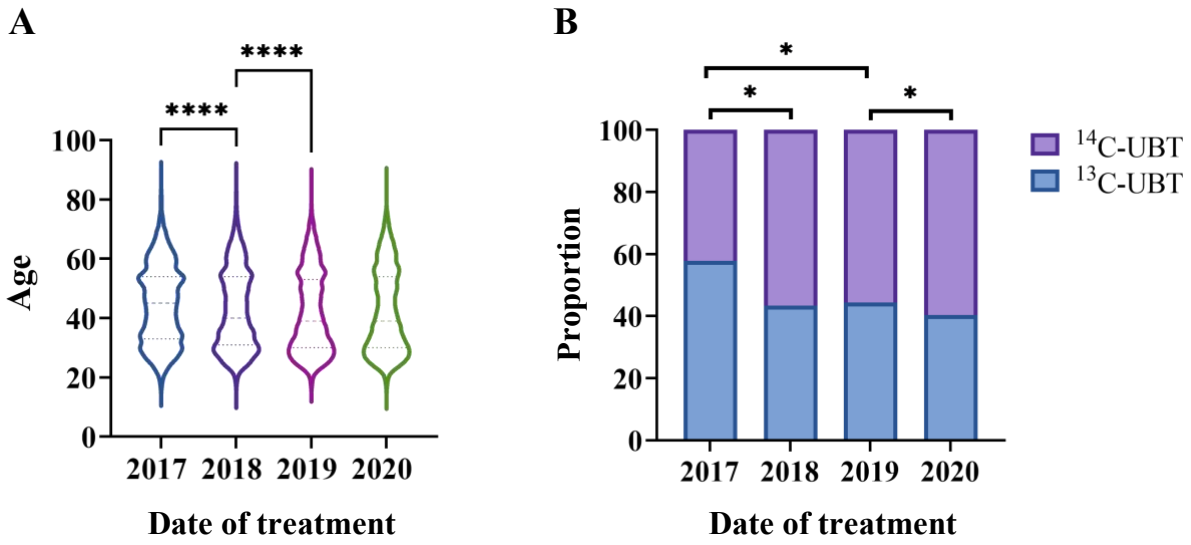
1. had *H. pylori* eradication treatments before
2. changed the regimen during therapy
3. clinical data were incomplete

Patients' data were deidentified and two researchers checked the data independently. Variables included age, sex, year, prescribed treatment and outcomes.

# Supplementary File 2. First-line Bismuth-containing quadruple therapy for *H. pylori* infection category pools and Proton pump inhibitor categories

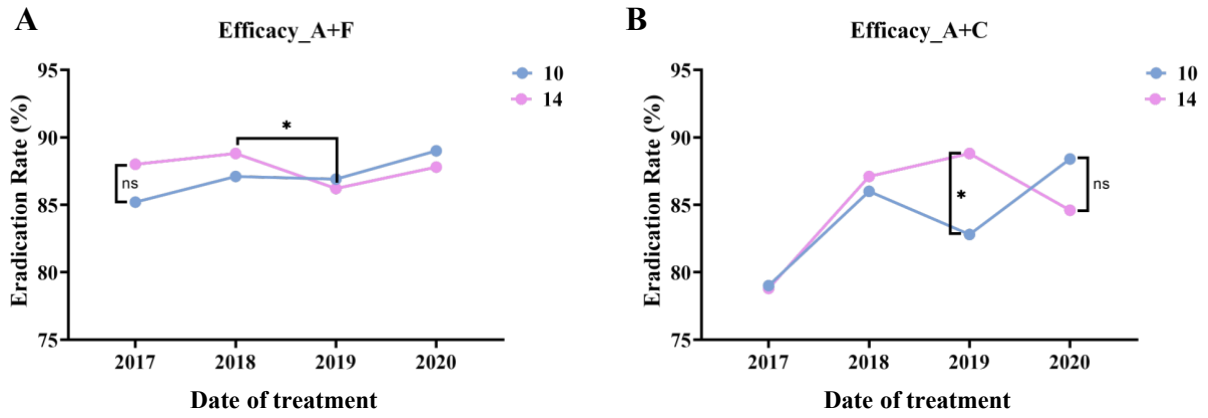
1. PPI + bismuth + A + F
2. PPI + bismuth + A + C
3. PPI + bismuth + F + C
4. PPI + bismuth + A + L
5. PPI + bismuth + F + L
6. PPI + bismuth + C + L
7. Other regimens (including different first-line therapies with frequencies lower than 0.5%)

Standard dose PPI including rabeprazole 10 mg (or 20 mg), pantoprazole 40 mg, esomeprazole 20 mg, omeprazole 20 mg and lansoprazole 30mg. Bismuth-containing quadruple therapy is defined as a PPI together with two antibiotics and bismuth salts given in the standard way. A, amoxicillin; C, clarithromycin; F, furazolidone; L, levofloxacin.



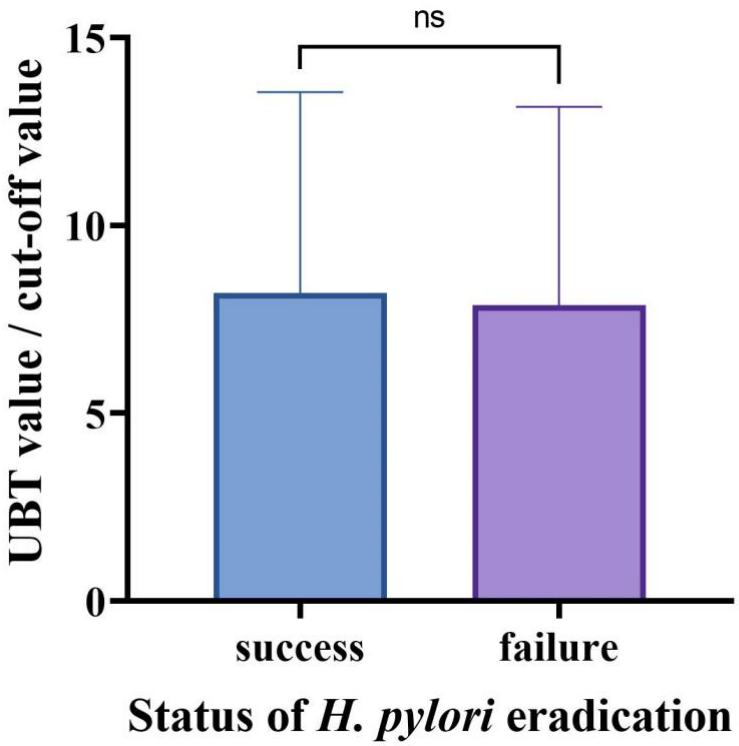

**Supplement Figure 1. Temporal trend analysis (2017–2020).**

(A) Trends in the age of patients. (B) Trends in the assessments after treatments.



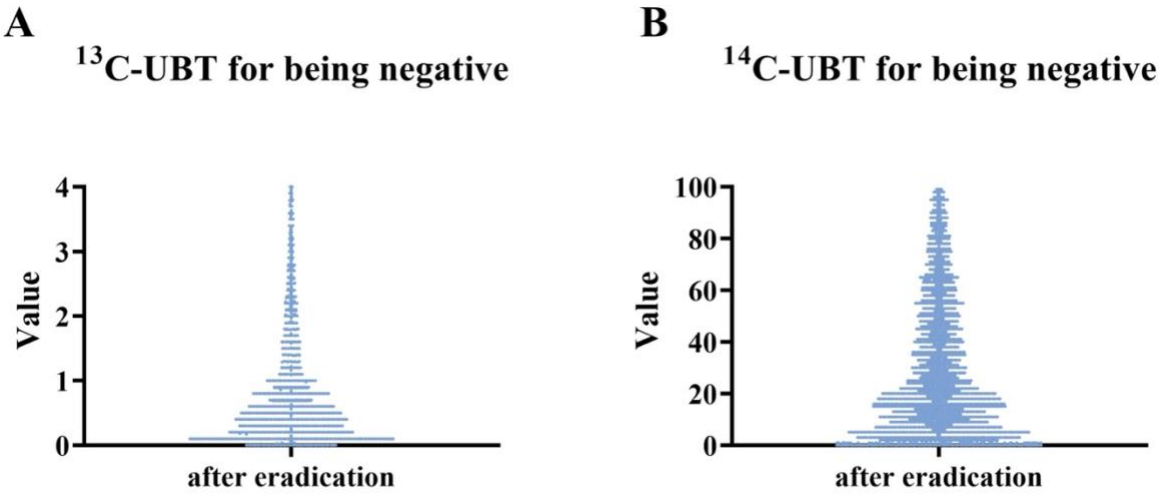

**Supplement Figure 2. Efficacy Results by treatment duration.**

(A) The eradication rate of regimens with amoxicillin and furazolidone. (B) The eradication rate of regimens with amoxicillin and clarithromycin.




**Supplement Figure 3. The association between UBT value before treatments and the outcomes.**

Data is standardized by dividing the UBT value by the cut-off value in different tests. The cut-off value of $^{13}$C-UBT was 4.0‰ (delta over baseline, DOB), and that of $^{14}$C-UBT was 100 (disintegrations per minute, DPM).




**Supplement Figure 4. Results of UBT for being negative after *H. pylori* eradication.**

(A) The scatter plot of $^{13}$C-UBT after *H. pylori* eradication. (B) The scatter plot of $^{14}$C-UBT after *H. pylori* eradication. The cut-off value of $^{13}$C-UBT was 4.0‰ (delta over baseline, DOB), and that of $^{14}$C-UBT was 100 (disintegrations per minute, DPM).

**Supplement Table 1. Effectiveness of first-line treatments per year.**

| Year | N | Success | Eradication rate (%) |
|---|---|---|---|
| 2017 | 3957 | 3317 | 83.8% |
| 2018 | 6486 | 5605 | 86.4%* |
| 2019 | 7568 | 6506 | 86.0%* |
| 2020 | 5459 | 4736 | 86.8%* |

* $P<0.05$ vs 2017.

**Supplement Table 2. Effectiveness of different first-line treatments per year.**

| Year | Regimen | N | Success | Eradication rate (%) |
|---|---|---|---|---|
| 2017 | A + F | 2785 | $2424_{a}$ | 87.0% |
| | A + C | 639 | $504_{b}$ | 78.9% |
| | F + C | 279 | $176_{c}$ | 63.1% |
| | A + L | 97 | $83_{a, b}$ | 85.6% |
| | F + L | 109 | $95_{a, b}$ | 87.2% |
| | C + L | 28 | $15_{c}$ | 53.6% |
| | Others | 20 | $20_{a, b}$ | 100.0% |
| 2018 | A + F | 4830 | $4275_{a}$ | 88.5% |
| | A + C | 863 | $750_{a}$ | 86.9% |
| | F + C | 498 | $330_{b, c}$ | 66.3% |
| | A + L | 110 | $96_{a}$ | 87.3% |
| | F + L | 122 | $107_{a}$ | 87.7% |
| | C + L | 22 | $11_{c}$ | 50.0% |
| | Others | 41 | $36_{a, b}$ | 87.8% |
| 2019 | A + F | 5009 | $4344_{a}$ | 86.7% |
| | A + C | 1853 | $1611_{a}$ | 86.9% |
| | F + C | 495 | $373_{b}$ | 75.4% |
| | A + L | 81 | $67_{a, b}$ | 82.7% |
| | F + L | 93 | $86_{a}$ | 92.5% |
| | C + L | 27 | $17_{b}$ | 63.0% |
| | Others | 10 | $8_{a, b}$ | 80.0% |
| 2020 | A + F | 4160 | $3664_{a}$ | 88.1% |
| | A + C | 827 | $712_{a}$ | 86.1% |
| | F + C | 397 | $298_{b}$ | 75.1% |
| | A + L | 21 | $18_{a, b}$ | 85.7% |
| | F + L | 34 | $30_{a, b}$ | 88.2% |
| | C + L | 18 | $13_{a, b}$ | 72.2% |
| | Others | 2 | $1_{a, b}$ | 50.0% |

Each subscript letter (a or b) denotes a subset of year categories whose column proportions do not differ significantly from each other at the .05 level. A, amoxicillin; C, clarithromycin; F, furazolidone; L, levofloxacin.

**Supplement Table 3. Recurrence after confirmation of H. pylori eradication with stricter criteria.**

| | Test | N | Standardized positivity | Recurrence rate |
|---|---|---|---|---|
| overall | overall | 1457 | 32 | 2.2% |
| | $^{13}$C-UBT | 843 | 16 | |
| | $^{14}$C-UBT | 751 | 16 | |
| A+F | overall | 1192 | 21 | 1.8% |
| | $^{13}$C-UBT | 636 | 9 | |
| | $^{14}$C-UBT | 556 | 12 | |
| A+C | overall | 242 | 5 | 2.1% |
| | $^{13}$C-UBT | 126 | 4 | |
| | $^{14}$C-UBT | 116 | 1 | |

Standardized positivity was defined by over 10‰ in $^{13}$C-UBT and over 250 in $^{14}$C-UBT. A, amoxicillin; C, clarithromycin; F, furazolidone.

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

| | Item No | Recommendation | Page No |
|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found | 1, 2 |
| **Introduction** | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 4, 5 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 5 |
| **Methods** | | | |
| Study design | 4 | Present key elements of study design early in the paper | 6 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 6 |
| Participants | 6 | (*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up | 6 |
| | | (*b*) For matched studies, give matching criteria and number of exposed and unexposed | Not applicable |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 6 |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 6 |
| Bias | 9 | Describe any efforts to address potential sources of bias | 6 |
| Study size | 10 | Explain how the study size was arrived at | 6 |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 6,7 |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding | 6,7 |
| | | (*b*) Describe any methods used to examine subgroups and interactions | 6,7 |
| | | (*c*) Explain how missing data were addressed | 6 |
| | | (*d*) If applicable, explain how loss to follow-up was addressed | 6 |
| | | (*e*) Describe any sensitivity analyses | Not applicable |
| **Results** | | | |
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | 8 |
| | | (b) Give reasons for non-participation at each stage | 8 |
| | | (c) Consider use of a flow diagram | 8 |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | 8 |
| | | (b) Indicate number of participants with missing data for each variable of interest | 8 |
| | | (c) Summarise follow-up time (eg, average and total amount) | 8 |
| Outcome data | 15* | Report numbers of outcome events or summary measures over time | 8-11 |

| | | | |
|---|---|---|---|
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included<br>(*b*) Report category boundaries when continuous variables were categorized<br>(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | 8-11<br>8-11<br>Not applicable |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | 10-11 |
| **Discussion** | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 15 |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 15 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 12-15 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | 15 |
| **Other information** | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 16 |

*Give information separately for exposed and unexposed groups.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.