scientific reports

Check for updates

OPEN

Randomized multicenter trial comparing minocycline and ornidazole with classical quadruple therapy in *Helicobacter pylori* treatment

Yi Lin[1,2,3], Xueyan Lin[1,2,3], Biao Suo[4], Qiuzhao Chen[4], Xianxing Cheng[5], Zhihui Lin[1,2,3] & Xueping Huang[1,2,3]✉

This study evaluated the efficacy, safety, and cost of minocycline, ornidazole, esomeprazole, and bismuth (MOEB) therapy versus classical therapy (amoxicillin, clarithromycin, esomeprazole, and bismuth potassium citrate, ACEB) for Helicobacter pylori eradication. In a randomized trial of 390 patients, MOEB demonstrated superior eradication rates (93.2% per-protocol, 78.5% intention-to-treat) compared to ACEB (82.5% per-protocol, 72.8% intention-to-treat). Adverse events were significantly lower with MOEB (19.3% vs. 33.8%, $p = 0.0019$). MOEB was also more cost-effective, with a direct cost of 675.7 CNY versus 970.1 CNY for ACEB, yielding an incremental cost-effectiveness ratio of -27.5 CNY per eradication rate. MOEB is a safe, effective, and cost-efficient first-line regimen for *H. pylori* eradication.

Keywords *Helicobacter pylori*, Minocycline, Ornidazole, Quadruple therapy

Helicobacter pylori (*H. pylori*) is a well-established etiological agent of various gastric disorders, including chronic gastritis, gastroduodenal ulcers, and gastric cancer. Eradication of *H. pylori* is critical for preventing and managing these conditions[1]. Despite its widespread prevalence, symptomatic gastrointestinal disease develops in only 25–30% of infected individuals[2] Recently, *H. pylori* gastritis has been recognized as an infectious disease and included in the 11th Revision of the International Classification of Diseases (ICD-11), emphasizing the importance of treatment for all infected patients[2].

Effective treatments for *H. pylori* are urgently needed as eradication efficacy declines due to rising antibiotic resistance[3]. In China, resistance rates to clarithromycin, metronidazole, and levofloxacin have been increasing[4]. Primary resistance rates are reported as 20–50% for clarithromycin, 40–70% for metronidazole, and 20–50% for levofloxacin[4–6]. Geographic variability exists, impacting treatment outcomes with clarithromycin-containing triple therapy achieving only 43% success against clarithromycin-resistant strains[7]. Consequently, bismuth-containing quadruple therapy (BQT) is recommended as first-line treatment for *H. pylori* eradication. The Maastricht IV consensus in 2012 endorsed a regimen comprising a proton pump inhibitor (PPI), tetracycline, metronidazole, and bismuth[8]. The Maastricht VI/Florence consensus further recommends BQT as the primary treatment in areas with high clarithromycin resistance (> 15%) or where resistance status is unknown[9].

However, widespread adoption of BQT is hindered by challenges in accessing tetracycline in many countries, including China, and concerns over adverse events associated with tetracycline and the high metronidazole resistance rates[10,11]. Minocycline, a second-generation semi-synthetic tetracycline derivative, offers improved pharmacokinetic properties and efficacy against various pathogens. It boasts nearly 100% oral bioavailability, is unaffected by food intake, and requires once or twice daily dosing, enhancing patient adherence[10,12,13]. Moreover, minocycline demonstrates good sensitivity against *H. pylori* and higher safety, akin to tetracycline, with limited reports of secondary resistance development post-treatment failure[6,14,15]. Ornidazole, a newer nitroimidazole

[1]The Shengli Clinical Medical College, Fujian Medical University, Fuzhou 350001, China. [2]Fuzhou University Affiliated Provincial Hospital, Fuzhou 350001, China. [3]Gastroenterology and Hepatology Department, Fujian Provincial Hospital, Fuzhou 350001, China. [4]Department of Gastroenterology, Xiamen Hospital of Traditional Chinese Medicine, Xiamen 361000, China. [5]Department of Gastroenterology, Wuyishan Municipal Hospital, Nanping 353000, China. ✉email: hxuep@mail2.sysu.edu.cn

derivative, exhibits prolonged gastric retention due to its extended half-life, coupled with potent bactericidal activity against anaerobic bacteria and fewer side effects compared to metronidazole[16]. Its convenience of once or twice daily dosing orally further supports patient compliance.

Collectively, these attributes suggest that minocycline and ornidazole present promising alternatives to tetracycline and metronidazole in *H. pylori* eradication therapy. Despite their potential benefits, studies on their combined use for *H. pylori* eradication are limited, and current guidelines do not advocate for the use of semi-synthetic tetracyclines in *H. pylori* treatment[2,9,17]. Therefore, the primary objective of this study is to evaluate whether the eradication efficacy of minocycline, ornidazole, esomeprazole, and bismuth (MOEB) as a first-line treatment regimen is not inferior to the classic regimen of amoxicillin, clarithromycin, esomeprazole, and bismuth (ACEB) for *H. pylori* infection. Additionally, the study aims to assess the incidence of adverse effects, patient compliance, and the cost-effectiveness of the treatments.

Results

Subjects

In this randomized study, 390 subjects with *H. pylori* infection, who were treatment-naive, were assigned to either the MOEB group ($n=195$) or the ACEB group ($n=195$) for the ITT analysis (Fig. 1). Of the assigned subjects, 166 in the MOEB group and 175 in the ACEB group met the endpoint and were included in the mITT analysis after excluding 29 subjects from the MOEB group and 20 from the ACEB group due to adverse events or loss of follow-up. The PP analysis was performed on 161 subjects in the MOEB group and 171 subjects in the ACEB group, after removing 5 non-compliant subjects from the MOEB group and 4 from the ACEB group who did not consume at least 85% of the prescribed medication (Fig. 1). The baseline characteristics and endoscopic findings of the two groups were comparable and showed no significant differences (Table 1).

Eradication rates

In the PP analysis, the lower bound of the 95% CI for difference between MOEB groups was greater than the pre-established non-inferiority margin of -10% (95%CI 3.3–18.2%, $p=0.0041$). The same CI was derived with the ITT population (Table 2).

The PP eradication rates of the MOEB group and the ACEB group were 93.2% (150/161) and 82.5% (141/171), and the ITT eradication rates were 78.5% (153/195) and 72.8% (142/195), respectively. In the mITT analysis, the eradication rates were 92.2% (153/166) with three of five subjects of low compliance and negative UBT results in the MOEB group, and 81.1% (142/175) with one of four subjects of low compliance and negative UBT results in the ACEB group.

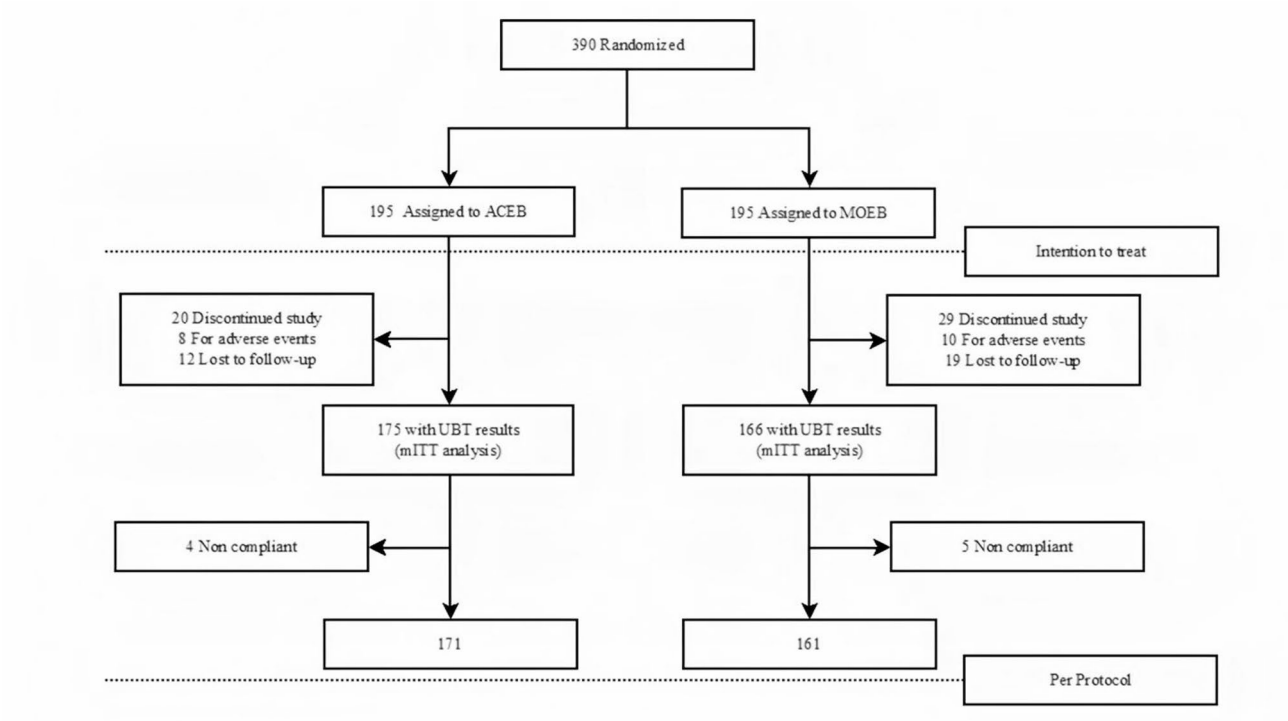

Fig. 1. Flowchart of participants. ACEB, amoxicillin, clarithromycin, esomeprazole, and bismuth therapy; MOEB, minocycline, ornidazole, esomeprazole, and bismuth therapy; ITT, intention-to-treat; mITT, modified intention-to-treat; PP, per-protocol; UBT, urea breath test.

	ACEB($n=195$)	MOEB($n=195$)	*P*-Value
Gender, n (%)			0.155
Male	98	83	
Female	97	112	
Age (mean ± SD), year	44.13 ± 13.940	42.38 ± 13.010	0.202
BMI, kg/m^2	22.86 ± 2.820	23.21 ± 2.844	0.220
Smoking, n (%)	39	41	0.900
Alcohol, n (%)	26	24	0.880
Tea, n (%)	48	58	0.306
Coffee, n (%)	35	31	0.686
Diagnosis, n (%)			0.141
Peptic ulcer disease	26	16	
Non-ulcer or uninvestigated dyspepsia	169	179	

Table 1. Baseline characteristics of the subjects. SD, standard deviation.

Analysis	MOEB group	ACEB group	95% CI for difference between MOEB and ACEB groups	*p*-Value
ITT	78.5% (153/195)	72.8% (142/195)	5.6%	0.238
95% CI	72.7-84.2%	66.6-79.1%	-3.1-14.5%	
PP	93.2% (150/161)	82.5% (141/171)	10.7%	0.004
95% CI	89.3-97.1%	76.8-88.2%	3.3-18.2%	
mITT	92.2% (153/166)	81.1% (142/175)	11.0%	0.004
95% CI	88.1-96.3%	75.4-86.9%	3.5-18.7%	

Table 2. Eradication rate of each group. ITT, intention-to-treat; MITT, modified intention-to-treat; PP, per-protocol; MOEB, minocycline, ornidazole, esomeprazole, and bismuth potassium citrate therapy; ACEB, amoxicillin, clarithromycin, esomeprazole, and bismuth potassium citrate therapy.

Adverse effects, *n* (%)	ACEB($n=183$)	MOEB($n=176$)
Nausea/Vomiting	22(12.0%)	7(4.0%)
Anorexia	8(4.4%)	1(0.6%)
Taste distortion	17(9.3%)	1(0.6%)
Diarrhea/	3(1.64%)	1(0.6%)
Constipation	2(1.1%)	5(2.8%)
Abdominal pain	1(0.5%)	0(0%)
Abdominal discomfort	4(2.2%)	6(3.4%)
Fatigue	0(0%)	1(0.6%)
Headache/Dizziness	13(7.1%)	12(6.8%)
Patients with adverse effects	62(33.9%)	34(19.3%)
Discontinued medications because of adverse effects	8(4.4%)	10(5.7%)
Good compliance	171(93.4%)	161(91.5%)

Table 3. Adverse effects and compliance. Twelve patients lost to follow-up in ACEB group, and 19 patients lost to follow-up in MOEB therapy group are excluded from the assessment of adverse effects and compliance.

Compliance

The complete follow-up rate was 85.1% (166/195) in the MOEB group and 89.7% (175/195) in the ACEB group. In the completed follow-up cases, 96.9% (161/166) of subjects in the MOEB group and 97.7% (171/175) in the ACEB group took more than 85% of the prescribed medicine. No significant difference was observed between the two groups in terms of compliance ($p=0.745$) (Table 3).

Adverse events

Out of 176 patients in the MOEB group, 34 (19.3%) experienced at least one adverse event, while 62 out of 183 patients (33.8%) in the ACEB group experienced the same. The adverse event rate was significantly lower in the MOEB group compared to the ACEB group ($P=0.0019$). The most common adverse events in the MOEB group were headache/dizziness (12/176, 6.8%) and nausea/vomiting (7/176, 4.0%), while in the ACEB group, the most frequent adverse events were nausea/vomiting (22/183, 12.0%) and taste distortion (17/183, 9.3%) (Table 3).

Variables, *n*/*N* (%)	ACEB (175)	MOEB (166)
Gender		
Female	89	96
Male	86	70
Age		
35	56	51
35–55	70	88
55	49	27
Body mass index		
18.0	9	5
18.0-23.9	112	87
24.0	54	74
Cigarette smoking		
Yes	33	37
No	142	129
Alcohol drinking		
Yes	20	23
No	155	143
Tea drinking		
Yes	39	55
No	136	111
Coffee drinking		
Yes	28	31
No	147	135
Diagnosis		
PUD	25	11
Non-ulcer or UD	150	155
Compliance		
Good	171*($p = 0.024$)	161*($p = 0.014$)
Poor	4	5

Table 4. Univariate analysis of the factors that influence the efficacy of Helicobacter pylori eradication using modified bismuth quadruple therapy for PP. *$P < 0.05$. Twenty patients (Twelve lost to follow-up, eight intolerants to medications) in the ACEB group and twenty-nine patients (nineteen lost to follow-up and ten intolerants to medications) in the MOEB therapy group who do not have urea breath test results after treatment are excluded from the assessment. NA, not applicable; PUD, peptic ulcer disease; UD, uninvestigated dyspepsia.

Group	Cost (CNY)	Effectiveness (%)	C/E	ΔC/ΔE
ACEB	970.1	82.5	11.76	
MOEB	675.7	93.2	7.25	-27.5

Table 5. Cost-effectiveness analysis. CNY, Chinese yuan; Effectiveness is evaluated by Helicobacter pylori eradication rate; ΔC, incremental cost between ACEB and MOEB therapy; ΔE, incremental effectiveness between ACEB and MOEB therapy.

Predictors of successful *H. pylori* eradication

Univariate analysis showed that the rates of *H. pylori* eradication were significantly higher among compliant patients compared to non-compliant patients in both the MOEB (OR 0.106, 95%CI (0.014, 0.772), $p = 0.014$) and ACEB (OR 0.071, 95%CI (0.007, 0.705), $p = 0.024$) groups. No significant effects were observed for gender, age, body mass index, cigarette smoking, alcohol consumption, tea consumption, coffee consumption, or a diagnosis of ulcers (Table 4).

Cost-efficiency analyses

The direct cost of the MOEB regimen was 675.7 CNY, while the cost of the ACEB regimen was 970.1 CNY, both for a 14-day course of therapy. The eradication rates for the MOEB and ACEB regimens were 93.2% and 82.5%, respectively. The cost per eradication rate was 7.25 CNY and 11.76 CNY, respectively. The incremental cost-effectiveness ratio (ΔC/ΔE) was − 27.5 CNY per eradication rate (Table 5).

Discussion

This study showed that the MOEB regimen was effective in eradicating *H. pylori* as a first-line therapy, with 195 cases in the ITT analysis, 166 cases in the mITT analysis, and 161 cases in the PP analysis. According to the eradication rate categorization of *H. pylori* infection treatments proposed by Graham et al.[18], in the ITT analysis, the *H. pylori* therapies was divided into five categories: A (≥95%), B (90-94%), C (85-89%), D (81-84%), and F (≤80%), which represented "excellent," "good," "acceptable," "poor," and "unacceptable," respectively. Meanwhile, in the PP analysis, the categories were A (≥95%), B (90-94%), C (86-89%), and F (≤85% eradication), which represented "excellent," "good," "poor," and "unacceptable," respectively. the MOEB regimen was classified as acceptable (ITT analysis) and good (PP analysis).

In recent years, the issue of increasing drug resistance in *H. pylori* has made eradication efforts more challenging. A study conducted in Beijing found high resistance rates in *H. pylori* to commonly used antibiotics such as amoxicillin (4.4%), clarithromycin (52.6%), metronidazole (54.8%), levofloxacin (63.4%), and tetracycline (7.3%)[4]. Due to this high resistance, current domestic and international consensus[9,17] does not support the use of standard triple or non-bismuth quadruple regimens containing clarithromycin and metronidazole. Instead, amoxicillin or tetracycline-bismuth quadruple regimens are recommended, with amoxicillin being the preferred choice due to its low resistance rate. However, a significant number of patients (15–24%) have documented penicillin allergy, making the use of amoxicillin limited[19–22]. Tetracycline- or clarithromycin-based bismuth quadruple therapy is recommended for patients with penicillin allergy[17]. However, the increasing drug resistance of clarithromycin has made it less effective, while the high drug resistance rate of metronidazole and significant side effects of tetracycline also limit their clinical application. This study explores the potential use of minocycline in place of tetracycline and ornidazole instead of metronidazole, as a new regimen aimed at achieving better eradication results.

A comprehensive literature search in PubMed revealed a scarcity of reports on minocycline-containing regimens for *H. pylori* eradication. Prior to 2010, Realdi et al.[23] and Murakami et al.[24] reported an eradication rate of approximately 40% with minocycline-containing triple therapy as first-line treatment. As a result, triple therapy with semisynthetic tetracyclines is not recommended for *H. pylori* infection treatment. Subsequently, the efficacy of a bismuth-containing quadruple therapy based on minocycline was explored. Ierardi et al.[25] investigated the efficacy of minocycline-containing quadruple therapy as third-line rescue therapy. The study randomly divided 54 patients into two groups: rabeprazole, rifabutin, minocycline, and bismuth (RRMB) or rabeprazole, tinidazole, minocycline, and bismuth (RTMB) for 10 days. The ITT and PP analyses revealed an eradication rate of 77.7% and 84.0% for RRMB therapy, respectively, while only 51.9% were eradicated in both ITT and PP analyses with RTMB therapy. These results suggest that the eradication effect of a bismuth quadruple regimen containing minocycline and metronidazole may not be ideal, which could be due to high drug resistance among the study subjects or a 10-day treatment course. Song et al.[26] evaluated the effectiveness of the esomeprazole, minocycline, metronidazole, and bismuth quadruple therapy (EMMB) as first-line and second-line treatments. They found that the EMMB therapy had an 85.5% eradication rate in the ITT analysis and 92.6% in the PP analysis for first-line treatment, and 82.8% in ITT analysis and 89.5% in PP analysis for second-line treatment. Zhang et al.[27] conducted a study on the efficacy of minocycline-containing quadruple therapy as a first-line rescue treatment. They randomly divided 360 patients into three groups: a minocycline/amoxicillin (RMAB) group, a minocycline/metronidazole (RMMB) group, and an amoxicillin/clarithromycin (RACB) group. All groups received a combination of rabeprazole and bismuth for 14 days, creating a quadruple regimen. According to the ITT analysis, the RMAB group had an eradication rate of 85.7%, the RMMB group had a rate of 77.1%, and the RACB control group had a rate of 71.7%. The PP analysis showed that the RMAB group had an eradication rate of 89.5%, the RMMB group had a rate of 84.3%, and the RACB group had a rate of 76.8%. Several studies have also indicated a high eradication rate (78.0–83.8%) with minocycline-based bismuth quadruple therapy, which is not inferior to other regimens. This trend was also supported by a recent meta-analysis[28].

The 2022 Chinese national clinical practice guideline on *H. pylori* eradication treatment[29] recommends the use of bismuth quadruple therapy (BQT) for primary and secondary eradication of *H. pylori* infection for 14 days. The Maastricht VI/Florence consensus[9] recommends that if individual susceptibility testing is not available, the first-line treatment in areas of high (>15%) or unknown clarithromycin resistance should be BQT. An analysis of first-line empirical treatments in 21,533 patients from 27 European countries showed that triple therapy with amoxicillin and clarithromycin was most commonly prescribed (39%), achieving an 81.5% modified intention-to-treat eradication rate. Over 90% eradication was obtained only with 10-day bismuth quadruple or 14-day concomitant treatments[30].

The European Registry on *H. pylori* management (Hp-EuReg) recently analyzed the effectiveness and safety of single-capsule BQT in real-world use in European countries (mostly Spain, Italy, and Portugal). The study included 2,100 cases: the modified intention-to-treat efficacy achieved was 94.6% (95% CI 93.2–95.8%) in first-line therapy, 89.3% (95% CI 86.2–92.3%) in second-line therapy, and 91.9% (95% CI 79.5–88.4%) as rescue treatments from third to sixth line[31]. Even in areas with a high prevalence of metronidazole resistance, the combination of PPI, bismuth, metronidazole, and tetracycline for 10–14 days achieved an ≥85% eradication rate[32,33]. Pylera® (bismuth, metronidazole, and tetracycline) is a three-in-one capsule formulation of this combination aimed at reducing pill burden, with a >90% success rate in over 5,000 patients in clinical practice[31].

Recently, a meta-analysis involving 30 studies (6,482 patients) evaluated the efficacy and safety of BQT with Pylera® plus PPI to eradicate and showed that the intention-to-treat efficacy was 90% (95% CI 87–92%, 21 studies) in first-line therapy, 89% (95% CI 86–93%, 12 studies) in second-line therapy, and 82% (95% CI 78–87%, nine studies) in third-line therapy, with no differences between the type or dosage of PPI used[34].

Given the rising global *H. pylori* resistance to metronidazole, coupled with the challenges in accessing tetracycline and its frequent use, our study aimed to explore alternative options to metronidazole and tetracycline

in the classic bismuth quadruple therapy. We proposed ornidazole and minocycline as substitutes and conducted a comprehensive evaluation of the eradication rate and safety profile of the modified MOEB regimen. Our study revealed that the MOEB regimen achieved eradication rates of 78.5% (95% CI 88.5–96.5%) in the intention-to-treat (ITT) analysis and 93.2% (95% CI 89.3–97.1%) in the per-protocol (PP) analysis. Importantly, no serious adverse events were reported, underscoring the viability of this alternative therapeutic approach in effectively eradicating.

According to the 2022 Chinese national clinical practice guideline on *H. pylori* eradication treatment, the recommended first-line therapy consists of a 14-day bismuth-based regimen, with the following antibiotic combinations endorsed: amoxicillin/clarithromycin, amoxicillin/levofloxacin, tetracycline/metronidazole, amoxicillin/metronidazole, and amoxicillin/tetracycline[29]. Utilizing the same PPI and bismuth components as MOEB, the direct medication costs (based on quotes provided by pharmaceutical retailer[35]) for these regimens are respectively: 970.1 CNY (amoxicillin/clarithromycin), 634.1 CNY (amoxicillin/levofloxacin), 448.0 CNY (tetracycline/metronidazole), 501.5 CNY (amoxicillin/metronidazole), and 505.9 CNY (amoxicillin/tetracycline), whereas our MOEB regimen costs 675.6 CNY. In our study, MOEB not only demonstrated a higher eradication rate compared to the amoxicillin/clarithromycin regimen but also featured lower expenses, along with favorable safety and compliance profiles. A randomized controlled trial (RCT) by Wei Fu et al.[36] involving 400 patients reported an eradication rate of 89.8% and an adverse event rate of 20.1% for the quadruple regimen of amoxicillin/levofloxacin as first-line therapy. Malfertheiner P. et al. et al.'s multicenter phase III study[37] found an eradication rate of 93% and an adverse event rate of 47% for the tetracycline/metronidazole regimen. Choe J.W. et al.'s study[38] revealed an eradication rate of 96.6% and an adverse event rate of 23% for amoxicillin/metronidazole as a first-line therapy. A meta-analysis by Lv Z.F. et al. et al.[39] indicated an eradication rate of 89.0% and an adverse event rate of 18.9% for amoxicillin/tetracycline as first-line *H. pylori* treatment. Compared with the currently recommended first-line therapies, MOEB offers a moderate cost alternative with a high eradication rate and a lower incidence of adverse effects, particularly considering the convenience of its twice-daily dosing regimen in contrast to the four times daily regimen required for regimens containing tetracycline or metronidazole.

These findings are in line with previous studies. It has been reported both domestically and internationally that the drug resistance rate of metronidazole is high[40,41], and there is cross-resistance with ornidazole. However, some studies have shown that increasing the dosage and frequency of metronidazole can partially overcome its resistance in bismuth-containing quadruple therapy[8,22,42]. Ornidazole has a longer half-life and can remain in the stomach for an extended period of time, making it necessary to take it orally only once or twice daily. Ornidazole also has a stronger inhibition and killing effect on most anaerobic bacteria and fewer side effects than metronidazole[16]. Bismuth has been shown to increase the eradication rate of *H. pylori*-resistant strains by 30–40%[43]. As a result, the study showed that the quadruple therapy of minocycline combined with ornidazole had a high eradication rate, achieving better efficacy than the quadruple therapy of minocycline combined with metronidazole and the traditional quadruple therapy of amoxicillin combined with clarithromycin.

The study showed that drug compliance (excluding lost follow-up cases) was 97.7% in the MOEB group, which is comparable to that of the ACEB group (96.9%). The high compliance was related to the twice-daily administration frequency. In this research, despite the fact that approximately 19.3–33.8% of patients experienced adverse effects, the majority of these were mild to moderate in nature, and only a small number of patients discontinued treatment due to unbearable side effects. Dizziness and nausea were the predominant adverse reactions observed in patients receiving the minocycline and ornidazole-based bismuth quadruple regimen. Dizziness is believed to be primarily due to the reversible vestibular reaction of minocycline, presenting as vertigo, tinnitus, and ataxia, accompanied by nausea. Vomiting and other vestibular dysfunctions often occur at the start of drug administration and can be resolved after 24–48 h of cessation. In this study, all patients in the observation group showed no significant symptoms of dizziness after 24 h of cessation. Nausea, which may be attributed to ornidazole, abated soon after cessation. These findings are consistent with other studies that have shown that minocycline-containing regimens have a high level of safety[24,25,44].

The attrition rates observed in this study (14.9% in MOEB vs. 10.3% in ACEB) align with prior multicenter trials evaluating *H. pylori* eradication therapies. For instance, the European Registry on *H. pylori* management (Hp-EuReg) reported attrition rates ranging from 12 to 18% in real-world studies involving over 21,000 patients, reflecting challenges inherent to longitudinal antibiotic trials[30,31]. Notably, sensitivity analyses (ITT vs. PP) demonstrated consistent superiority of MOEB over ACEB across all populations, suggesting minimal attrition-related bias. The slightly higher ITT-PP discrepancy in the MOEB group (14.7% vs. ACEB's 9.7%) primarily stemmed from a higher proportion of discontinuations due to adverse events (10 vs. 8 cases) and loss to follow-up (19 vs. 12 cases). This pattern mirrors findings by Ierardi et al.[25], where attrition disproportionately impacted ITT outcomes in regimens with higher adverse event rates. Importantly, baseline characteristics remained balanced between groups, mitigating concerns about selection bias. While attrition is a recognized limitation in antibiotic trials, our results underscore the robustness of MOEB's efficacy, particularly given its favorable safety profile (19.3% adverse events vs. 33.8% in ACEB, $p = 0.0019$) and cost-effectiveness (direct cost: 675.7 CNY vs. 970.1 CNY). These advantages position MOEB as a viable alternative, especially in regions with limited access to tetracycline or rising clarithromycin resistance[9,29].

This clinical trial was limited by several factors. Firstly, the routine culture of *H. pylori* was not performed, precluding the evaluation of the eradication rate of minocycline- and/or ornidazole-susceptible *H. pylori* strains. This study did not routinely conduct *H. pylori* resistance testing, a design choice that aligns with current clinical practices and guideline recommendations in China. According to the 2022 Chinese Clinical Guidelines for *H. pylori* Infection Treatment[29], antibiotic susceptibility testing is not routinely recommended for patients undergoing initial treatment but is considered only after treatment failure. This recommendation stems from the low prevalence of resistance testing in primary healthcare institutions (< 10%) and the robust evidence

supporting the broad efficacy of empirical bismuth-containing quadruple therapy (BQT)[9,29]. Nevertheless, we fully acknowledge the significant impact of antibiotic resistance on eradication rates and have referenced Fujian Province's resistance surveillance data to substantiate our findings. Recent research by our team revealed that *H. pylori* resistance rates in Fujian were notably higher for clarithromycin (43.9%), metronidazole (40.98%) and amoxicillin (11.22%) compared to tetracycline (4.88%)[45]. This context elucidates the superiority of the MOEB regimen over ACEB—minocycline, a tetracycline derivative, exhibits an extremely low resistance rate in China (0.7-8.2%)[46], while ornidazole, with its prolonged half-life (12–14 h) and gastric retention properties, may partially overcome metronidazole resistance[16,47]. Which consistent with the 93.2% per-protocol (PP) eradication rate observed in the MOEB group of this study. Furthermore, bismuth enhances the eradication rate of resistant strains by 30–40% through mechanisms such as disrupting bacterial biofilms and inhibiting efflux pumps[43], further bolstering the robustness of the MOEB regimen. Although individualized resistance data are lacking, our findings are highly consistent with real-world data from regions with high resistance rates (e.g., the 94.6% PP eradication rate of single-capsule BQT in the Hp-EuReg study)[31], validating MOEB as a viable empirical first-line regimen. Future research should incorporate regional resistance surveillance to optimize personalized treatment strategies. Secondly, no subgroup analysis was conducted to determine the resistance status of ornidazole. Finally, endoscopy was not mandatory for the study participants, leading to some inter-individual variations.

Conclusion

This study demonstrated that the quadruple regimen comprising bismuth, minocycline, and ornidazole exhibited comparable eradication rates to the classical treatment of *H. pylori* patients, which consists of amoxicillin and clarithromycin. Moreover, the quadruple regimen was deemed safe and may be recommended as a treatment option for patients with penicillin allergies who are infected with *H. pylori*.

Materials and methods

Study design and participants

A multicenter, randomized, open-label, parallel-design clinical trial was conducted from July 2021 to May 2022 at three hospitals across Fujian, China. The study received approved from the Ethics Committee of Fujian Provincial Hospital (K2020-06-009) and adhered to Good Clinical Practices (GCP) and the Declaration of Helsinki. Written informed consent was obtained from all participants before enrollment.

The inclusion criteria for the study were: (1) adult patients (> 18 years old) diagnosed with *H. pylori* infection using one of the following methods: a positive rapid urease test, histologic evidence of *H. pylori* by modified Giemsa staining, or a positive 14 C/13 C-urea breath test; (2) patients who had not taken any drugs that could potentially affect the study results (such as PPIs, H2 blockers, mucosal protective agents, antibiotics, or traditional Chinese medicine) for the preceding four weeks; and (3) patients who were informed and gave their consent to participate in the study, with complete medical and follow-up records.

The exclusion criteria were: (1) prior eradication therapy for *H. pylori*; (2) severe dysfunction of the heart, lung, liver, or kidney, or low immunity; (3) prior gastric surgery; (4) advanced gastric cancer or other malignancy or gastrointestinal hemorrhage; (5) any specific contraindications or allergic reactions to the study drugs; (6) mental disorders or communication difficulties; (7) pregnancy or lactation or refusal to use an appropriate method of contraception throughout the study; or (8) any other condition that might affect the evaluation of the clinical results, as determined by the principal investigator or sub-investigator.

This study protocol has been registered with the Chinese Clinical Trials Registry (ChiCTR2000033717(10/06/2020)).

Randomization and treatment allocation

Patients were randomly assigned to one of two therapy groups through computer-generated randomization. The first group received the MOEB regimen, which consisted of minocycline (100 mg twice daily), ornidazole (500 mg twice daily), esomeprazole (20 mg twice daily), and bismuth (220 mg twice daily), for a duration of two weeks. The second group received the ACEB regimen, which consisted of amoxicillin (1000 mg twice daily), clarithromycin (500 mg twice daily), esomeprazole (20 mg twice daily), and bismuth (220 mg twice daily), for a duration of two weeks.

Six weeks after completing the therapy, the success of *H. pylori* eradication was determined through a negative $^{14}C/^{13}C$-urea breath test. The drug compliance and adverse events were evaluated by a physician through direct questioning. Compliance was considered satisfactory if drug intake exceeded 85%. Patients who had drug compliance below 85% or experienced a serious adverse drug reaction or gastrointestinal bleeding during the study treatment were not allowed to continue in the study.

Assessment of *H. pylori* infection

Invasive testing

To establish the presence of an active *H. pylori* infection, biopsy specimens were taken from the antrum's greater curvature and the upper part of the gastric corpus. These specimens were fixed in formalin for further evaluation of *H. pylori* infection using Giemsa staining and rapid urease test (RUT Kit; Delta West, Bentley, Australia).

$^{14}C/^{13}C$-urea breath test (UBT)

Subjects were required to fast for 4 h prior to the test. A predose breath sample was collected, followed by the oral administration of 100 mg of 14 C/13 C-urea powder (UCBT Kit; Atom High Tech, Beijing, China) dissolved in 100 mL of water. A second breath sample was then collected 20 min later. The cutoff value was set at 2.5%. The

collected samples were analyzed using an isotope ratio mass spectrometer (GIRMS ZC-202; Wan Yi Sci & Tech, Anhui, China).

Endpoints

The assessment of *H. pylori* eradication was performed 6 weeks post-therapy completion using the $^{14}C/^{13}C$-urea breath test (UBT). In order to prevent interference with the test results, the use of proton pump inhibitors, H2 blockers, mucosal protective agents, antibiotics, traditional Chinese medicine, and anti-urease drugs such as ecabet sodium was prohibited 4 weeks prior to the UBT.

Our primary efficacy outcome was *H. pylori* eradication, established by negative urea breath tests at least 28 days after the end of treatment (week 10 visits) in the PP population, who fully adhered to the study protocol and excluded those with poor compliance. The ITT analysis included all eligible patients enrolled in the study who received at least one dose of the therapy, while the mITT analysis comprised all patients who received one dose of therapy and had an endpoint measure. The percentage of patients with drug compliance greater than 85%, the incidence of adverse events, and cost-effectiveness between the two therapy groups were also assessed.

Sample size

Based on a preliminary experiment, the eradication rates of the MOEB and ACEB regimens were found to be 92% and 81%, respectively, with a rate difference of around 10%. With a power of 90% and a two-sided type 1 error rate of 5%, a sample size of 195 subjects per treatment arm was calculated to account for a 10% loss to follow-up.

Statistical analysis

Comparative non-inferiority of the two groups was assessed through hypothesis testing (Fisher's exact test) and the derivation of a two-sided 95% confidence interval (CI) using the Newcombe/Wilson score with continuity correction. The equivalence margin was set at -10%. The per-protocol (PP) population was used as the primary analysis set. However, the eradication rates were also evaluated in the intention-to-treat (ITT) and modified intention-to-treat (mITT) populations. In the ITT analysis, all subjects who received treatment were included, while in the mITT analysis, only those who received at least one dose of the drug and had an endpoint measurement were considered. The PP analysis only included subjects who had taken at least 85% of the prescribed drugs. A cost-effectiveness analysis was conducted to determine the direct medical costs. The effectiveness of the strategies was assessed by considering the eradication rate (E) and the cost of the regimens (C) in CNY 2023, and the resulting incremental cost-effectiveness ratios ($\Delta C/\Delta E$).

Data analysis was performed using SPSS 25.0 (IBM Corp., Armonk, NY, United States), while GraphPad Prism7.0 was used for mapping. Measurement data with a normal distribution were expressed as mean ± SD and analyzed using Student's t-test or analysis of variance (ANOVA). For non-normally distributed data, the median and interquartile range were used, and analyzed by the Mann-Whitney U test or Kruskal-Wallis test. Categorical variables were analyzed using Pearson's chi-square test or Fisher's exact test and presented as numbers (percentages). Univariate and multivariate logistic regression analyses were conducted to identify independent risk factors, and odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. A two-sided *p*-value of less than 0.05 was considered statistically significant and the Bonferroni correction method was used for *p*-value adjustment in multiple comparisons.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request. All of the individual participant data collected during the trial, after deidentification and study protocol will be shared beginning 1 months and ending 12 months following article publication. Researchers who provide a methodologically sound proposal and to achieve aims in the approved proposal could send proposals to hxuep@mail2.sysu.edu.cn to gain access.

Received: 8 January 2025; Accepted: 5 May 2025
Published online: 10 May 2025

References

1. McColl, K. E. Clinical practice. Helicobacter pylori infection. *N Engl. J. Med.* **362** (17), 1597–1604 (2010).
2. Sugano, K. et al. Kyoto global consensus report on Helicobacter pylori gastritis. *Gut* **64** (9), 1353–1367 (2015).
3. Malfertheiner, P. et al. Management of Helicobacter pylori infection-the Maastricht V/Florence consensus report. *Gut* **66** (1), 6–30 (2017).
4. Zhang, Y. X. et al. Primary antibiotic resistance of Helicobacter pylori strains isolated from patients with dyspeptic symptoms in Beijing: a prospective serial study. *World J. Gastroenterol.* **21** (9), 2786–2792 (2015).
5. Su, P. et al. Antibiotic resistance of Helicobacter pylori isolated in the Southeast coastal region of China. *Helicobacter* **18** (4), 274–279 (2013).
6. Bai, P. et al. Susceptibility of Helicobacter pylori to antibiotics in Chinese patients. *J. Dig. Dis.* **16** (8), 464–470 (2015).
7. Nyssen, O. P. et al. Sequential versus standard triple first-line therapy for Helicobacter pylori eradication. *Cochrane Database Syst. Rev.* **2016** (6), CD009034 (2016).
8. Malfertheiner, P. et al. Management of Helicobacter pylori infection–the Maastricht IV/ Florence consensus report. *Gut* **61** (5), 646–664 (2012).
9. Malfertheiner, P. et al. *Management of Helicobacter pylori infection: the Maastricht VI/Florence consensus report*. *Gut*, (2022).
10. O'Connor, J. P., Taneike, I. & O'Morain, C. Improving compliance with helicobacter pylori eradication therapy: when and how? *Th. Adv. Gastroenterol.* **2** (5), 273–279 (2009).
11. Gisbert, J. P. Rescue regimens after Helicobacter pylori treatment failure. *World J. Gastroenterol.* **14** (35), 5385–5402 (2008).

12. Chopra, I. & Roberts, M. *Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance*. *Microbiol. Mol. Biol. Rev.*, **65**(2): p. (2001). 232 – 60; second page, table of contents.
13. Barza, M. & Schiefe, R. T. Antimicrobial spectrum, Pharmacology and therapeutic use of antibiotics. Part 1: tetracyclines. *Am. J. Hosp. Pharm.* **34** (1), 49–57 (1977).
14. Smith, K. & Leyden, J. J. Safety of Doxycycline and Minocycline: a systematic review. *Clin. Ther.* **27** (9), 1329–1342 (2005).
15. Horiki, N. et al. Annual change of primary resistance to clarithromycin among Helicobacter pylori isolates from 1996 through 2008 in Japan. *Helicobacter* **14** (5), 86–90 (2009).
16. Munoz, E., Castella, J. & Gutierrez, J. F. Vivo and in vitro sensitivity of Trichomonas gallinae to some nitroimidazole drugs. *Vet. Parasitol.* **78** (4), 239–246 (1998).
17. Chinese Society of. [Fifth Chinese National consensus report on the management of Helicobacter pylori infection]. *Zhonghua Nei Ke Za Zhi*. **56** (7), 532–545 (2017).
18. Graham, D. Y., Lu, H. & Yamaoka, Y. A report card to grade Helicobacter pylori therapy. *Helicobacter* **12** (4), 275–278 (2007).
19. Unger, N. R., Gauthier, T. P. & Cheung, L. W. Penicillin skin testing: potential implications for antimicrobial stewardship. *Pharmacotherapy* **33** (8), 856–867 (2013).
20. Lee, C. E. et al. The incidence of antimicrobial allergies in hospitalized patients: implications regarding prescribing patterns and emerging bacterial resistance. *Arch. Intern. Med.* **160** (18), 2819–2822 (2000).
21. Solensky, R., Earl, H. S. & Gruchalla, R. S. Penicillin allergy: prevalence of vague history in skin test-positive patients. *Ann. Allergy Asthma Immunol.* **85** (3), 195–199 (2000).
22. Raja, A. S. et al. The use of penicillin skin testing to assess the prevalence of penicillin allergy in an emergency department setting. *Ann. Emerg. Med.* **54** (1), 72–77 (2009).
23. Realdi, G. et al. Pretreatment antibiotic resistance in Helicobacter pylori infection: results of three randomized controlled studies. *Helicobacter* **4** (2), 106–112 (1999).
24. Murakami, K. et al. Effectiveness of minocycline-based triple therapy for eradication of Helicobacter pylori infection. *J. Gastroenterol. Hepatol.* **21** (1 Pt 2), 262–267 (2006).
25. Ierardi, E. et al. Quadruple rescue therapy after first and second line failure for Helicobacter pylori treatment: comparison between two tetracycline-based regimens. *J. Gastrointestin Liver Dis.* **23** (4), 367–370 (2014).
26. Song, Z. Q. & Zhou, L. Y. Esomeprazole, Minocycline, metronidazole and bismuth as first-line and second-line regimens for Helicobacter pylori eradication. *J. Dig. Dis.* **17** (4), 260–267 (2016).
27. Zhang, L. et al. Application of Minocycline-Containing bismuth quadruple therapies as First-Line regimens in the treatment of Helicobacter pylori. *Gastroenterol. Res. Pract.* **2019**, p9251879 (2019).
28. Kocazeybek, B. & Tokman, H. B. Prevalence of primary antimicrobial resistance of H. pylori in Turkey: A systematic review. *Helicobacter* **21** (4), 251–260 (2016).
29. Zhou, L. et al. 2022 Chinese National clinical practice guideline on Helicobacter pylori eradication treatment. *Chin. Med. J. (Engl).* **135** (24), 2899–2910 (2022).
30. Nyssen, O. P. et al. European registry on Helicobacter pylori management (Hp-EuReg): patterns and trends in first-line empirical eradication prescription and outcomes of 5 years and 21 533 patients. *Gut* **70** (1), 40–54 (2021).
31. Nyssen, O. P. et al. European registry on Helicobacter pylori management: Single-capsule bismuth quadruple therapy is effective in real-world clinical practice. *United Eur. Gastroenterol. J.* **9** (1), 38–46 (2021).
32. Gisbert, J. P. & McNicholl, A. G. *Optimization strategies aimed to increase the efficacy of H. pylori eradication therapies*. *Helicobacter*, **22**(4). (2017).
33. Graham, D. Y. *Transitioning of Helicobacter pylori therapy from trial and error to antimicrobial stewardship*. *Antibiot. (Basel)*, **9**(10). (2020).
34. Nyssen, O. P., McNicholl, A. G. & Gisbert, J. P. Meta-analysis of three-in-one single capsule bismuth-containing quadruple therapy for the eradication of Helicobacter pylori. *Helicobacter* **24** (2), e12570 (2019).
35. *The Prices of Chinese Medicine. Available from*: https://pages.tmall.com/wow/yao/act/ziyinghome?from=zebra:*offline [Accessed October 30, 2023]*.
36. Fu, W. et al. Randomized clinical trial: Esomeprazole, bismuth, Levofloxacin, and amoxicillin or Cefuroxime as First-Line eradication regimens for Helicobacter pylori infection. *Dig. Dis. Sci.* **62** (6), 1580–1589 (2017).
37. Malfertheiner, P. et al. Helicobacter pylori eradication with a capsule containing bismuth subcitrate potassium, metronidazole, and Tetracycline given with Omeprazole versus clarithromycin-based triple therapy: a randomised, open-label, non-inferiority, phase 3 trial. *Lancet* **377** (9769), 905–913 (2011).
38. Choe, J. W. et al. Comparative study of Helicobacter pylori eradication rates of concomitant therapy vs modified quadruple therapy comprising proton-pump inhibitor, bismuth, amoxicillin, and metronidazole in Korea. *Helicobacter* **23** (2), e12466 (2018).
39. Lv, Z. F. et al. Meta-analysis: is combination of Tetracycline and amoxicillin suitable for Helicobacter pylori infection? *World J. Gastroenterol.* **21** (8), 2522–2533 (2015).
40. Zhang, W. et al. Bismuth, Lansoprazole, amoxicillin and metronidazole or clarithromycin as first-line Helicobacter pylori therapy. *Gut* **64** (11), 1715–1720 (2015).
41. Hsu, P. I. et al. Ten-day quadruple therapy comprising proton-pump inhibitor, bismuth, Tetracycline, and Levofloxacin achieves a high eradication rate for Helicobacter pylori infection after failure of sequential therapy. *Helicobacter* **19** (1), 74–79 (2014).
42. Fischbach, L. & Evans, E. L. Meta-analysis: the effect of antibiotic resistance status on the efficacy of triple and quadruple first-line therapies for Helicobacter pylori. *Aliment. Pharmacol. Ther.* **26** (3), 343–357 (2007).
43. Dore, M. P., Lu, H. & Graham, D. Y. Role of bismuth in improving Helicobacter pylori eradication with triple therapy. *Gut* **65** (5), 870–878 (2016).
44. Nakajima, S. et al. Multiple-antibiotic-resistant Helicobacter pylori infection eradicated with a tailor-made quadruple therapy. *J. Gastroenterol. Hepatol.* **27** (Suppl 3), 108–111 (2012).
45. Huang, X. et al. Antibiotic resistance profile of Helicobacter pylori to 14 antibiotics: a multicenter study in Fujian, China. *PeerJ* **11**, e15611 (2023).
46. Zhou, K. et al. Minocycline in the eradication of Helicobacter pylori infection: A systematic review and meta-analysis. *World J. Gastroenterol.* **30** (17), 2354–2368 (2024).
47. Lehmann, F. S. et al. Effect of ornidazole and clarithromycin resistance on eradication of Helicobacter pylori in peptic ulcer disease. *Aliment. Pharmacol. Ther.* **14** (3), 305–309 (2000).

Author contributions

Yi Lin wrote the figures and tables, and Xueyan Lin and Yi Lin wrote the manuscript. Biao Suo, Qiuzhao Chen, and, Xianxing Chen collected the clinical data. Zhihui Lin revised the draft. Xueping Huang designed the clinical trial. Zhihui Lin and Xueping Huang made the analysis.

Funding

This study is supported by Fujian Provincial Natural Science Foundation, Grant No.2022J011025, No.2020J05264, and, No.2023J011210 & Science and Technology Planning Project of Fujian Provincial Health Commission,

Grant No. 2020GGA002 and 2022QNA003.

Declarations

Competing interests
The authors declare no competing interests.

Ethical approval
This study protocol has been registered with the Chinese Clinical Trials Registry (Registration number: ChiCTR2000033717, Date of registration: 10/06/2020) and was approved by the Ethics Committee of Fujian Provincial Hospital (K2020-06-009).

Additional information
Correspondence and requests for materials should be addressed to X.H.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

© The Author(s) 2025