A Comparative Study of Sequential Therapy and Standard Triple Therapy for *Helicobacter pylori* Infection: A Randomized Multicenter Trial

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OBJECTIVES: **Studies conducted in large populations of patients and providing full information on *Helicobacter pylori* (*H. pylori*) antibiotic resistance are needed to determine the efficacy of sequential therapy (SQT) against this pathogen. This study compared eradication rates with SQT and standard triple therapy (STT), and evaluated the impact of antibiotic resistance on outcomes.**

METHODS: **The study population included adults with positive *H. pylori* culture presenting at four centers in China between March 2008 and December 2010. Patients were randomly assigned to 10 days of treatment with esomeprazole, amoxicillin, and clarithromycin (STT; $n=140$) or to 5 days of treatment with esomeprazole and amoxicillin, followed by 5 days of esomeprazole, clarithromycin, and tinidazole (SQT; $n=140$). Eradication was assessed 8–12 weeks after treatment.**

RESULTS: **There was no significant difference between the eradication rates achieved with STT (66.4% (95% confidence interval (CI) 59.3–74.3)) and SQT (72.1% (65.0–79.3); $P=0.300$) in either the intention-to-treat analysis or the per-protocol analysis (72.7% (65.6–79.7) and 76.5% (69.7–83.3), respectively; $P=0.475$). Clarithromycin resistance (CLA-R, odds ratio (OR)=8.34 (3.13–22.26), $P<0.001$) and metronidazole resistance (MET-R, OR=7.14 (1.52–33.53), $P=0.013$) both independently predicted treatment failure in the SQT group. Patients in the SQT group with dual CLA-R and MET-R had a lower eradication rate (43.9%) than those with isolated CLA-R (88.9%, $P=0.024$) or isolated MET-R (87.8%, $P<0.001$).**

CONCLUSIONS: ***H. pylori* eradication rates with STT and SQT were compromised by antibiotic resistance. SQT may be suitable in regions with high prevalence of isolated CLA-R, but it is unsatisfactory when both CLA-R and MET-R are present.**

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INTRODUCTION

Helicobacter pylori (*H. pylori*) infection is an important causal factor in a wide range of upper gastrointestinal diseases, including chronic gastritis, peptic ulcer disease, and gastric cancer. As such, it is a major contributor that affects public health on a worldwide basis, especially in developing countries (1).

Traditionally, standard triple therapy (STT) comprising a proton pump inhibitor (PPI) combined with two antibiotics (clarithromycin and amoxicillin or metronidazole) is the first-line option for empiric *H. pylori* eradication (2,3). However, the effectiveness of STT has declined to unacceptable levels in many regions of the world, mainly owing to antibiotic resistance. Consequently, it is no longer a suitable first-line treatment approach (4), and there is a clear need for new regimens with good efficacy and safety in order to effectively manage this widespread form of infection.

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Sequential therapy (SQT), consisting of 5 days of dual therapy (PPI plus amoxicillin) followed by 5 days of triple therapy (PPI plus clarithromycin plus tinidazole or metronidazole), has recently attracted widespread attention. Several systematic reviews (5–8) concluded that SQT is associated with a good eradication efficacy and safety, and showed that it was more effective than STT. On the basis of this evidence, SQT appears to offer an effective therapeutic option to replace STT for first-line treatment of *H. pylori*. However, as the majority of evidence supporting the use of SQT comes from Italy (9–13), publication bias may compromise the available data. Studies undertaken in Spain (14), Korea (15), and Latin America (16) failed to replicate the initial encouraging results from Italian centers, whereas data from Taiwan (17) support the use of SQT over STT. Distinct geographical variations of *H. pylori* antibiotic resistance are likely to explain these divergent results.

To date, relatively few trials of SQT eradication provided *H. pylori* culture results, and antibiotic susceptibility data are not generally available for most of the enrolled cases (9,10,17–25). The small populations with antibiotic resistance data included in clinical trials have impeded the evaluation of the effects of antibiotic resistance on eradication efficacy. Clinical trials with larger populations of patients that provide complete antibiotic resistance data are needed to investigate the efficacy of SQT in populations with different levels and/or different patterns of antibiotic resistance. This information will enable populations susceptible to SQT to be defined that, in turn, will improve eradication rates and will avoid antibiotic resistance.

In China, where there is high prevalence of *H. pylori* infection and high resistance rates to clarithromycin and metronidazole, STT remains the most widely used treatment for *H. pylori* eradication (26). However, the suitability of SQT therapy for Chinese patients with *H. pylori* infection has not been investigated.

Therefore, the primary objective of this study was to compare the eradication rates of *H. pylori* infection associated with STT and SQT regimens. We also attempted to determine factors that influenced eradication efficacy and investigated the effects of different patterns of antibiotic resistance on *H. pylori* eradication.

METHODS

Study design and patients

The trial was undertaken between March 2008 and December 2010. Consecutive patients (18 to 75 years of age) with dyspepsia who had been referred for upper endoscopy at four tertiary hospitals in Beijing, Shanghai, Wuhan, and Guangzhou were included in the study. All patients recruited into the study had positive *H. pylori* cultures, but none had previously received treatment for *H. pylori* infection.

Patients who had received PPIs, H_2-receptor blockers, bismuth salts, or antibiotics during the previous 4 weeks were excluded from entering the study. None of the patients had a history of gastrointestinal malignancy, previous gastric or esophageal surgery, Zollinger–Ellison syndrome, and severe concomitant cardiovascular, respiratory, hematological, renal, hepatic, or neurological diseases. The study also excluded women who were pregnant or lactating, as well as those known to be allergic to any of the study drugs.

The protocol was approved by the Ethics Committees of the four hospitals involved in the study. The study was conducted according to the principles of the Declaration of Helsinki and the standards of Good Clinical Practice. All patients provided informed consent to participate in the study.

Study design

The patients were interviewed to obtain demographic data and medical history, and were randomly assigned to one of two treatment groups. A computer-generated randomization scheme (SAS version 8.0; SAS Institute, Cary, NC), with stratification by center, was constructed using a block design (block size of four) by an independent statistician and was used to determine treatment allocation. Allocation was concealed by the use of opaque envelopes that were opened by the investigator when the patient was eligible for the study and had provided written informed consent.

The patients were randomly assigned to treatment in a 1:1 ratio within 2 weeks of a positive culture result. STT comprised 20 mg of esomeprazole, 500 mg of clarithromycin, and 1 g of amoxicillin taken twice a day for 10 days. SQT comprised 20 mg of esomeprazole and 1 g of amoxicillin taken twice a day for 5 days, followed by 20 mg of esomeprazole, 500 mg of clarithromycin, and 500 mg of tinidazole taken twice a day for 5 days. Treatment allocation was not blinded.

Patients were asked to return within 1–3 days after treatment to assess compliance with therapy and to determine the incidence of side effects. *H. pylori* detection and determination of the outcome of therapy was undertaken 8–12 weeks after treatment.

Drugs that could influence the study results were prohibited during the study.

No follow-up was performed as part of this study. Patients with failed eradication were conventionally treated with a second eradication regimen based on the culture and resistance tests performed in this study.

H. pylori detection

All eligible patients underwent upper endoscopy upon entry into the study. A gastric biopsy taken from the antrum was subjected to a rapid urease test (RUT; HPUT-H102, San Qiang Bio & Che, Fujian, China). If the result was positive, two additional specimens (from the antrum and corpus) were obtained for *H. pylori* culture. Patients with positive cultures were classified as being infected with *H. pylori*.

Patients with peptic ulcer and macroscopic gastric mucosal erosions underwent repeat upper endoscopy to determine the outcome of eradication therapy 8–12 weeks after the end of treatment. Three gastric biopsies were taken at this visit: one from the antrum for RUT testing and one each from the antrum and corpus for Warthin–Starry staining. *H. pylori* eradication was defined by the demonstration of a negative RUT test and a negative Warthin–Starry stain.

A ^{13}C-urea breath test (UBT, UCBT Kit, Atom High Tech, Beijing, China) was used to confirm the presence of *H. pylori* infection in patients without peptic ulcer or macroscopic gastric mucosal erosions at 8–12 weeks after treatment. *H. pylori* infection was considered to be eradicated if the UBT result was negative.

The endoscopists, pathologists, and technicians who performed RUT and UBT were all blinded to the treatment group allocation. PPIs, H_2-receptor blockers, bismuth salts, or antibiotics were discontinued for at least 4 weeks before post-treatment testing.

H. pylori strain collection and antibiotic susceptibility testing

H. pylori strains were isolated from gastric mucosal samples, and *in vitro* antibiotic resistance was tested by the E-test (27). The antibiotic resistance breakpoints were ≥1.0 μg/ml for amoxicillin and clarithromycin and ≥8 μg/ml for metronidazole.

CYP2C19 polymorphism

CYP2C19 polymorphism was analyzed to characterize PPI metabolism. The genotyping of the two mutated genes of CYP2C19 was performed using real-time PCR. DNA was extracted from gastric mucosal samples using a QIA amp Mini Kit (Qiagen, Düsseldorf, Nordrhein-Westfalen, Germany). Two pairs of probes were designed to distinguish the wild and mutated alleles of the *CYP2C19* gene. The specificity of the probes was validated using the sequence method. Patients were classified into three groups according to the genotype by identifying the CYP2C19 wild-type (CYP2C19*1) gene and the two mutated alleles (CYP2C19*2 and CYP2C19*3). Those without mutation (*1/*1) were designated as the homozygous extensive metabolizer group; those with one mutation (*1/*2 or *1/*3) were designated as the heterozygous extensive metabolizer group; and those with two mutations (*2/*2, *3/*3, or *2/*3) were designated as the poor metabolizer group.

Tolerability and compliance

Adverse events were evaluated by using open-ended questions, by patient self-reports, and from physical examinations.

Compliance, determined by pill counts, was defined as good when >90% of the prescribed drugs were taken. Patients who had taken 80% of the treatment drugs were considered to show poor compliance and were excluded from the per-protocol (PP) analysis.

Statistical analysis

The sample size calculation was based on results from a published pooled-data analysis (5). Based on a 93.4% eradication rate for SQT and a 79.6% eradication rate for 10-day STT, it was calculated that at least 126 patients per treatment arm would be required to provide 90% power to detect a statistically significant difference between treatments at a two-sided probability level of 95%. At least 139 patients were recruited into each group to account for a 10% withdrawal rate.

Statistical analysis was performed using SPSS for Windows (version 16, SPSS, Chicago, IL). The primary outcome variable was the *H. pylori* eradication rate with STT and SQT, evaluated using both intention-to-treat (ITT) and PP analyses. Secondary outcomes were eradication rates in subgroups of patients with clarithromycin resistance (CLA-R) and metronidazole resistance (MET-R). Isolated CLA-R was defined as clarithromycin resistance and susceptibility to metronidazole. Isolated MET-R was defined as metronidazole resistance and susceptibility to clarithromycin.

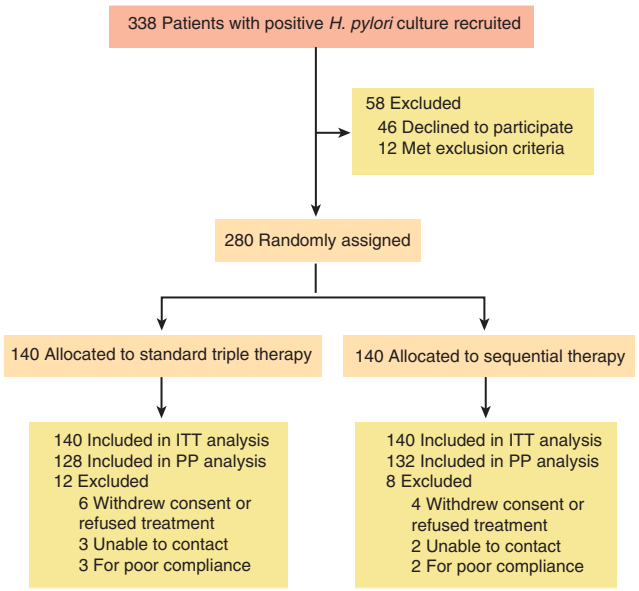

Figure 1. Patient flowchart. ITT, intention to treat, PP, per protocol.

Between-group differences were evaluated using Student's *t*-test for continuous variables and Pearson's χ^2 or Fisher's exact test for categorical variables. Host and bacterial parameters were analyzed using univariate analysis. Variables with a *P* value of <0.3 were included in a multivariate logistic regression analysis to identify independent factors for eradication outcome. Odds ratios (ORs) and 95% confidence intervals (CIs) for unsuccessful eradication were calculated in relation to the different variables. The frequency of adverse events and compliance was compared between regimens. The *P* values of <0.05 were considered to be statistically significant.

All authors had access to the study data and reviewed and approved the final manuscript.

RESULTS

Figure 1 shows patient flowchart, according to the CONSORT statement advice.

A total of 338 patients with positive *H. pylori* cultures were recruited into the study; 280 patients were enrolled and randomly assigned to receive STT or SQT therapy. The two treatment groups were well matched with respect to baseline demographic data, clinical characteristics, antibiotic resistance, and CYP2C19 polymorphisms (**Table 1**). A total of 20 patients were excluded from the PP analysis, 12 in the STT group and eight in the SQT group (*P*=0.353), because of poor compliance, withdrawal of consent, or because they were lost to follow-up.

In the ITT population, eradication rates were 66.4% in the STT group and 72.1% in the SQT group (*P*=0.300; **Table 2**). In the PP population, eradication rates were 72.7% with STT and 76.5% with

SQT therapy ($P=0.475$). **Table 3** presents the effects of amoxicillin resistance (AMO-R), CLA-R, and MET-R on the success of STT and SQT in the PP population, according to each possible combination of resistances. However, AMO-R was too rare, and we did not include it in our further analyses.

Patients with isolated CLA-R in the SQT group achieved a significantly higher eradication rate than in the STT group ($P=0.040$). In the SQT group, the eradication rate in patients with dual CLA-R and MET-R was significantly lower than in patients with isolated CLA-R ($P=0.024$), isolated MET-R ($P<0.001$), or dual susceptibility ($P<0.001$; **Figure 2**).

Univariate analysis indicated that the eradication rate was significantly higher in patients without CLA-R compared with those with CLA-R in both the STT ($P<0.001$) and SQT ($P<0.001$) groups. In the SQT group, the eradication rate was significantly higher in patients without MET-R compared with those with MET-R ($P=0.001$). There was no significant effect of age, gender, body mass index, smoking, endoscopic findings, amoxicillin resistance, or CYP2C19 polymorphism on eradication rates in either group (**Table 4**).

Table 1. Baseline characteristics

Variables	STT group (*n*=140)	SQT group (*n*=140)	*P* value
Age, mean±s.d. (years)	43.3±14.2	43.6±13.1	0.865
Gender (male/female)	71/69	61/79	0.231
BMI, mean±s.d. (kg/m^2)	22.5±3.3	22.7±2.6	0.630
Smoking (yes/no)[a]	22/118	17/123	0.388
Endoscopic findings (PUD/NUD)[b]	23/117	20/120	0.619
Amoxycillin resistance, *n* (%)	7 (5.0)	6 (4.3)	0.776
Clarithromycin resistance, *n* (%)	58 (41.4)	54 (38.6)	0.626
Metronidazole resistance, *n* (%)	91 (65.0)	96 (68.6)	0.526
CYP2C19 polymorphism (hom EM:het EM:PM)[c]	57:66:7	63:56:13	0.234

BMI, body mass index; het EM, heterozygous extensive metabolizer; hom EM, homozygous extensive metabolizer; NUD, nonulcer dyspepsia; PM, poor metabolizer; PUD, peptic ulcer disease; SQT, sequential therapy; STT, standard triple therapy.

[a]Smoking was defined as consumption of more than one pack of cigarettes a week during the previous 6 months.

[b]Patients with duodenal or/and gastric ulcer were considered as having peptic ulcer disease; those without ulcers were considered as nonulcer dyspeptic patients.

[c]Eighteen patients were excluded from the CYP2C19 polymorphism analyses because not enough DNA could be extracted from gastric mucosal samples.

Multivariate analysis identified CLA-R as an independent predictor of treatment failure in both the STT (OR=9.51, 95% CI: 3.75–24.11, $P<0.001$) and SQT (OR=8.34, 95% CI: 3.13–22.26, $P<0.001$) groups. The presence of MET-R was also a predictor of treatment failure in the SQT group (OR=7.14, 95% CI: 1.52–33.53, $P=0.013$).

Both treatments were well tolerated and no major side effects were reported. In total, 12 (8.6%) patients in the STT group and 10 (7.1%) in the SQT group complained of mild, self-limiting side effects ($P=0.657$). None of the side effects resulted in discontinuation of therapy. The side effects (alone or in combination) included nausea (five STT patients and three SQT), epigastric pain (three STT patients and four SQT), diarrhea (three patients in each group), itching (two STT patients and one SQT), headache (two STT patients and one SQT), constipation (two STT patients), and taste alteration (one SQT patient). Good compliance was achieved in 132 (94.3%) patients in the STT group and in 131 (93.6%) patients in the SQT group ($P=0.802$).

DISCUSSION

Our data confirm the previously reported, disappointing *H. pylori* eradication rates using STT (4). We also showed unexpectedly poor eradication rates using the SQT regimen for *H. pylori* in China, with the lowest cure rates (72.1% in ITT and 76.5% in PP analysis) reported to date. These findings challenge the recommendations of systematic reviews (5–8) based primarily on data from Italy that show that SQT is more effective than STT, indicating that the studies reviewed are not generally applicable to other regions of the world.

CLA-R is an important factor reducing the efficacy of SQT. Results from previous studies indicate that progressively increasing CLA-R resistance rates result in a corresponding decrease in eradication rates. On the basis of PP population, eradication rates of >85% have been reported in studies with CLA-R rates below 20% (9,10,17,19–23,25) compared with an eradication rate of 82.8% in a study where the CLA-R rate was 26.5% (18) and with 76.5% in the present study with a CLA-R rate of 38.6%.

Both univariate and multivariate analyses of our data identified CLA-R as a factor that reduced the efficacy of SQT. However, patients with isolated CLA-R still had a relatively high eradication rate of 88.9% that declined to 43.9% when dual CLA-R and MET-R were present. These findings indicate that dual resistance is a major factor affecting the outcome of SQT. Indeed, the high prevalence of dual resistance in our study (31.1% among the PP population) explained the poor eradication rates achieved in the population as a whole.

Table 2. Comparison of *Helicobacter pylori* eradication rates between standard triple and sequential therapy

Analysis	Standard triple therapy		Sequential therapy		*P* value
	Patients (*n*)	ER (%, 95% CI)	Patients (*n*)	ER (%, 95% CI)	
ITT	140	66.4, 59.3–74.3	140	72.1, 65.0–79.3	0.300
PP	128	72.7, 65.6–79.7	132	76.5, 69.7–83.3	0.475

CI, confidence interval; ER, eradication rate; ITT, intention to treat; PP, per protocol.

Table 3. Effect of amoxicillin, clarithromycin and metronidazole resistance on *Helicobacter pylori* eradication rates in the PP population

	STT group		SQT group	
PP population	Total *n*	Successful *n* (%)	Total *n*	Successful *n* (%)
AMO-S CLA-S MET-S	28	26 (92.9)	33	32 (97.0)
AMO-R CLA-S MET-S	1	0 (0)	0	—
AMO-S CLA-R MET-S	16	7 (43.8)	9	8 (88.9)
AMO-S CLA-S MET-R	43	39 (90.7)	47	41 (87.2)
AMO-R CLA-R MET-S	0	—	0	—
AMO-R CLA-S MET-R	3	2 (66.7)	2	2 (100)
AMO-S CLA-R MET-R	34	18 (52.9)	37	17 (45.9)
AMO-R CLA-R MET-R	3	1 (33.3)	4	1 (25.0)
Total	128	93 (72.7)	132	101 (76.5)

AMO, amoxicillin; CLA, clarithromycin; MET, metrodinazole; PP, per protocol; S, sensible; SQT, sequential therapy; STT, standard triple therapy; R, resistant.

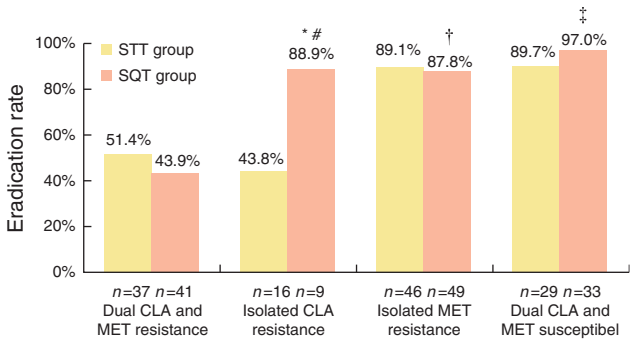

Figure 2. Effect of clarithromycin and metronidazole resistance on *Helicobacter pylori* eradication rates. *Isolated CLA resistance: STT group vs. SQT group, $P=0.040$. #SQT group: dual CLA and MET resistance vs. isolated CLA resistance, $P=0.024$. †SQT group: dual CLA and MET resistance vs. isolated MET resistance, $P<0.001$. ‡SQT group: dual CLA and MET resistance vs. dual CLA and MET susceptible, $P<0.001$. CLA, clarithromycin; MET, metronidazole; SQT, sequential therapy; STT, standard triple therapy.

The Maastricht IV Consensus Report (1) recommends prescribing SQT as a first-line regimen for *H. pylori* in regions with a high prevalence of CLA-R, when bismuth quadruple therapy is not available. Our results further illustrate that SQT is mainly suitable for areas with high isolated CLA-R prevalence and indicate that the efficacy of SQT is poor when dual CLA-R and MET-R are present.

It is generally thought that MET-R has relatively less impact on the outcome of SQT than CLA-R. Data on *H. pylori* eradication with SQT in isolated MET-R populations from previously published studies indicate that eradication rates are >85% (9,10,17,18,20,23), which is similar to our own findings. Both univariate and multivariate analyses of our data showed that *H. pylori* eradication rates with SQT in patients with MET-R were significantly lower than in those without MET-R, but the response difference was less marked than for CLA-R.

Although patients with isolated CLA-R achieved significantly higher cure rates with SQT than with STT, the differences in subgroups with dual resistance, isolated MET-R, and dual susceptibility failed to reach statistical significance. Thus, the higher eradication rates achieved in patients with isolated CLA-R explain the observed differences in efficacy between SQT and STT. Because of the relatively low proportion of patients with isolated CLA-R in our study, and its relatively low impact on the overall efficacy, we were unable to demonstrate any statistically significant differences in overall eradication rates between SQT and STT.

Recently, a study by Liou *et al.* (17) reported eradication rates of 87.0% (ITT) and 90.5% (PP) in Taiwanese patients with *H. pylori* infection who received at 10-day regimen of SQT. This response rate was significantly higher than that in our study. The reason for this difference is likely to be the different antibiotic resistance profiles in the two study populations. The CLA-R rate (10%) and MET-R rate (24%) in the study of Liou *et al.* (17) were significantly lower than those in our study. These observations indicate that SQT should not be used as an empirical regimen for first-line treatment of *H. pylori* infection, but should be considered as an optional therapeutic strategy for specific populations. In study of Liou *et al.* (17), extending the duration of treatment with SQT to 14 days resulted in a further 3–4% increase in eradication rates. Moreover, a 14-day SQT regimen was associated with a significantly higher eradication rate than the rate achieved with a 14-day STT regimen. Although a 14-day SQT regimen was not investigated in our study, it is unlikely that additional improvement would be seen because of the very limited efficacy seen in our study after 10 days.

This study provides data on the largest number of antibiotic-resistant patients exposed to SQT to date. In previous studies investigating antibiotic resistance in SQT, the maximum numbers of patients with CLA-R, MET-R, and dual resistance were 19, 46, and 10, respectively (9). Estimation of eradication rates in these small subpopulations of resistant patients is susceptible to the impact of random error. In our study, all patients had positive bacterial cultures and were residing in known regions of high resistance prevalence. The numbers of subpopulations with CLA-R, MET-R, and dual resistance were 50, 90, and 41 patients, respectively, which provided adequate sample sizes for evaluating the relationships between antibiotic resistance patterns and efficacy of SQT. However, AMO-R frequency was not high enough to allow us to perform any analysis on this resistance.

Table 4. Univariate analysis showing factors affecting *Helicobacter pylori* eradication

Variable	STT group (*n*=128)		SQT group (*n*=132)	
	Eradication, *n* (%)	*P* value	Eradication, *n* (%)	*P* value
Age				
<40 years	41 (69.5)	0.458	39 (72.2)	0.333
≥40 years	52 (75.4)		62 (79.5)	
Gender				
Male	51 (76.1)	0.357	45 (78.9)	0.566
Female	42 (68.9)		56 (74.7)	
BMI				
<22.0 kg/m^2	43 (70.5)	0.600	47 (79.7)	0.443
≥22.0 kg/m^2	50 (74.6)		54 (74.0)	
Smoking				
Yes	15 (71.4)	0.890	15 (88.2)	0.358
No	78 (72.9)		86 (74.8)	
Endoscopic findings				
PUD	18 (90.0)	0.058	18 (90.0)	0.158
NUD	75 (69.4)		83 (74.1)	
Amoxicillin resistance				
Yes	3 (42.9)	0.088	3 (50.0)	0.141
No	90 (74.4)		98 (77.8)	
Clarithromycin resistance				
Yes	26 (49.1)	<0.001	26 (52.0)	<0.001
No	67 (89.3)		75 (91.5)	
Metronidazole resistance				
Yes	60 (72.3)	0.899	61 (67.8)	0.001
No	33 (73.3)		40 (95.2)	
CYP2C19 polymorphism[a]				
Hom EM	38 (74.5)	0.438	47 (77.0)	0.520
Het EM	48 (76.2)		41 (78.8)	
PM	5 (100.0)		11 (91.7)	

BMI, body mass index; Het EM, heterozygous extensive metabolizer; Hom EM, homozygous extensive metabolizer; NUD, nonulcer dyspepsia; PM, poor metabolizer; PUD, peptic ulcer disease; SQT, sequential therapy; STT, standard triple therapy.

[a]Sixteen patients were excluded from the CYP2C19 polymorphism analysis because not enough DNA was extracted from gastric mucosal samples.

Our study also collected a comprehensive set of pretreatment baseline information. In addition to demographic data and clinical characteristics, complete antibiotic resistance rates and CYP2C19 polymorphisms were comparable in both treatment groups and added to the reliability of our findings.

Patients in our study who presented with peptic ulcer and gastric mucosal erosion received repeated endoscopies to observe the healing of mucosal lesions, and RUT and histological staining were used to determine whether *H. pylori* had been eradicated. For those patients without peptic ulcer and gastric mucosal erosion, a single UBT was used to determine the eradication results. This did not completely meet the recommendations of the Working Party of the European Helicobacter pylori Study Group for clinical trials (28), and may have introduced bias in our detection rates. However, for both practical and ethical reasons, few patients with nonulcer dyspepsia, especially those without mucosal lesions, are likely to give consent to participate in a trial that includes repeated endoscopies. For this reason, most studies perform only noninvasive post-treatment tests (mostly UBT). It should also be borne in mind that UBT has a sensitivity and specificity of >95%; therefore, using this single test after treatment is generally regarded as being an acceptable approach (29).

Although information on *H. pylori* antibiotic resistance was obtained at baseline, we chose to randomize patients into SQT and STT therapy groups in order to investigate the differences between SQT and STT regimens when used as first-line empiric therapy in Chinese patients. The results of *H. pylori* sensitivity tests were used to analyze the influence of antibiotic resistance on the efficacy of eradication, rather than to tailor the treatment regimen. When this study was designed and when the patients were being treated, STT was still recommended by the Chinese Gastrointestinal Association as the first-line empiric regimen, although some studies suggested that clarithromycin resistance could compromise the efficacy of STT.

We therefore believe that our results will help facilitate the selection of appropriate patients for SQT. Resistance patterns encountered in other areas of the world may already be similar to those in China or may become similar in the near future. In regions where there are high rates of CLA-R and MET-R, such as in our own study population, it is likely that eradication rates achieved with SQT for *H. pylori* infection will continue to become progressively more unsatisfactory. In contrast, in areas with high isolated CLA-R prevalence, SQT regimen remains a relatively good choice.

In conclusion, the eradication efficacies of both SQT and STT for *H. pylori* infection in China are poor, and this is mainly related to CLA-R and MET-R. The higher eradication rate with SQT compared with STT in the patients with isolated CLA-R indicates that SQT is mainly suitable for use in areas with high isolated CLA-R. The efficacy of SQT appears to be inadequate when CLA-R and MET-R coexist. Consequently, eradication of *H. pylori* infection remains a challenge, and alternative treatment regimens with good efficacy and safety are urgently needed.

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CONFLICT OF INTEREST

Guarantor of the article: Sanren Lin, BS.

Specific author contributions: Liya Zhou: study concept, study design, clinical studies, and manuscript editing; Jianzhong Zhang: study design and experimental studies; Minhu Chen, Xiaohua Hou, and Zhaoshen Li: clinical studies; Zhiqiang Song: clinical studies, data statistical analysis, and manuscript preparation; Lihua He: experimental studies; Sanren Lin: study concept, study design, and

manuscript editing. All authors have read and approved the final manuscript.

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Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Sequential therapy is associated with a high rate of eradication and is well tolerated. Therefore, it appears to offer an effective therapeutic option to replace standard triple therapy for first-line treatment of *H. pylori*.
- ✓ Sequential therapy can overcome clarithromycin resistance and is suitable for use in regions with a high prevalence of clarithromycin resistance, as recommended in the Maastricht IV Consensus.
- ✓ In previous trials, the small populations of patients with antibiotic resistance data have impeded the comprehensive evaluation of the effects of antibiotic resistance on the eradication efficacy of sequential therapy.

WHAT IS NEW HERE

- ✓ Sequential therapy is not suitable as first-line therapy for empiric *H. pylori* eradication, and it should be specifically selected according to the background pattern of antibiotic resistance.
- ✓ Antibiotic resistance is a major factor that influences the efficacy of sequential therapy: the prevalence of dual resistance to clarithromycin and metronidazole is the most critical factor that determines the eradication rate.
- ✓ The sequential regimen also exhibits relatively higher eradication efficacy than standard triple therapy when isolated clarithromycin resistance is detected.
- ✓ These findings indicate that sequential regimen is mainly suitable for use in regions and populations with a high prevalence of isolated clarithromycin resistance. This study also facilitates the understanding of the newly published Maastricht IV consensus.

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