Efficacy of quadruple therapy with clarithromycin based on faecal molecular antimicrobial susceptibility tests as first-line treatment for *Helicobacter pylori* infection: a protocol of a single-centre, single-blind, randomised clinical trial in China

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**ABSTRACT**

**Introduction** *Helicobacter pylori* is the most well-known risk factor for gastric cancer. Antibiotic resistance is the main reason for the failure of *H. pylori* eradication, and understanding the antibiotic resistance before treatment may be the main determinant of successful eradication of *H. pylori*. This study aims to evaluate the efficacy and safety of quadruple therapy based on faecal molecular antimicrobial susceptibility tests for the first-line eradication of *H. pylori* infection.

**Methods and analysis** This is a single-centre, single-blind, randomised controlled trial, enrolling 855 patients with *H. pylori* infection. Patients are randomised to three groups for a 14-day treatment: group A: amoxicillin- and clarithromycin-based bismuth-containing quadruple therapy (BQT) (rabeprazole 10 mg, amoxicillin 1 g, clarithromycin 500 mg and colloidal bismuth 200 mg two times per day); group B: clarithromycin medication history-based BQT (rabeprazole 10 mg, amoxicillin 1 g, furazolidone 100 mg (with clarithromycin medication history)/clarithromycin 500 mg (without clarithromycin medication history) and colloidal bismuth 200 mg two times per day); group C: antimicrobial susceptibility test-based BQT (rabeprazole 10 mg, amoxicillin 1 g, clarithromycin 500 mg (clarithromycin-resistant)/furazolidone 100 mg (clarithromycin resistant) and colloidal bismuth 200 mg two times per day). The primary end point is the eradication rate. The secondary end points are the incidence of adverse events and compliance.

**Ethics and dissemination** This study was approved by the Ethics Committee of Second Affiliated Hospital, School of Medicine, Zhejiang University (Number 20230103). The results will be published in the appropriate peer-reviewed journal.

**Trial registration number** NCT05718609.

**INTRODUCTION**

*Helicobacter pylori* was listed as a Class I carcinogen by the World Health Organization in 1994 and is closely associated with a variety of digestive tract diseases, such as chronic gastritis, peptic ulcer, gastric cancer and mucosa- assisted lymphoid tissue (MALT) lymphoma. About 90% of non-cardia gastric cancer is associated with *H. pylori* infection and the eradication of *H. pylori* makes intestinal gastric cancer a preventable disease.

Amoxicillin-clarithromycin-containing bismuth-containing quadruple therapy (BQT) is one of the most useful regimens against *H. pylori*. Though the eradication rate of amoxicillin-based and clarithromycin-based BQT is over 85% according to our previous study, the resistance to clarithromycin has been increasing in recent years and is considered a major cause of the failure of clarithromycin-based therapy. According to a systematic review and meta-analysis in...
2017, the prevalence of resistance to clarithromycin has risen significantly over time in the Asia-Pacific region in the past two decades, and the clarithromycin-containing regimens should be avoided in countries where the prevalence of clarithromycin resistance is higher than 20%. While the eradication rate for susceptibility-guided therapy with clarithromycin is quite promising, at >95%. Success of first-line eradication is likely to inhibit further bacterial resistance, decrease the difficulty of retreatment and reduce the medical burden, so it is better to eradicate *H. pylori* successfully at the initial treatment.

Nevertheless, because of the invasive examination to sampling, the economy–benefit ratio and the harsh cultivation conditions, traditional antimicrobial susceptibility test is rarely used before first-line eradication treatment in the real world. Molecular biology methods mainly used for the detection of clarithromycin-resistant genotypes of *H. pylori* have important guiding value for *H. pylori* eradication therapy.

Clarithromycin resistance is usually due to the mutation of A2142C/G and A2143G in *H. pylori*’s 23S ribosomal subunit RNA, which makes it possible to detect the resistance to clarithromycin using PCR kits or sequencing methods detecting 23S ribosomal RNA point mutations. In recent years, molecular assays for the detection of genotypic antibiotic resistance markers of *H. pylori* have been described, with many requiring endoscopically collected tissues and a few using non-invasively collected samples, which showed similar detection accuracy. Traditional molecular antimicrobial susceptibility tests via PCR-based assays directly from gastric mucosal tissues successfully bypass the need for culture but still require invasive collection methods. Compared with that, faecal antimicrobial susceptibility tests can inform and guide treatment choices by providing baseline antibiotic resistance status via antibiotic-associated single nucleotide polymorphisms (SNPs) in a rapid, accurate manner from non-invasively collected stool specimens, thus successfully obviating the need for endoscopy. Previous studies showed that molecular test using PCR kits in stool samples has good analytical sensitivity for the detection of clarithromycin-resistant *H. pylori*, with about 95% categorical agreement compared with agar dilution using *H. pylori* isolated from biopsy specimens. According to Zhou and Song’s study, the suitability of widespread use of first-line eradication therapy based on drug-sensitive results requires the eradication efficacy, efficiency–cost ratio, safety and the feasibility in clinical practice. More convenient, much safer and relatively non-expensive methods of sampling make it possible to popularise the antimicrobial susceptibility tests catering to both initial and salvage treatments.

What is more, the resistance to clarithromycin is also related to the medication history. Thus, to elevate the eradication rate and avoid multiple treatments, the eradication regimen tailored by knowledge of antibiotic medication history is recommended in the empiric eradication of *H. pylori* infection.

*H. pylori* remains highly sensitive to amoxicillin, furazolidone and tetracycline in China, especially East China. Antibiotic regimens with amoxicillin and furazolidone dominated in the past few years, as tetracycline was not available in our hospital pharmacy. Again, we choose furazolidone as an alternative to clarithromycin in this study. A meta-analysis stated that a 14-day furazolidone-containing regimen with a low daily dose of 200 mg was well tolerated and should be used as first-line treatment. Though Federal Drug Agency warns that furazolidone may reduce fertility or injure unborn children, the International Agency for Research on Cancer classified furazolidone into group 3, that is, not carcinogenic in humans. No serious adverse events (AEs) were reported among the cases in our previous large-scale retrospective study containing 25 796 patients. Furazolidone-containing therapies with a high eradication rate should be re-evaluated in other countries.

This essay described a protocol aimed to explore the efficacy, safety and economic benefits of the first-line therapy for *H. pylori* guided by faecal antimicrobial susceptibility tests. Our study might provide new evidence to inform the value of faecal antimicrobial susceptibility tests in first-line treatment to help to reduce therapeutic failure and the subsequent need for additional therapy courses, which in turn could result in decreased compliance, increased potential side effects and increased medical economic burden.

### METHODS AND ANALYSIS

We developed this protocol according to the Standard Protocol Items: Recommendations for Interventional Trials. The trial is registered at ClinicalTrials.gov and any important changes to the protocol will be implemented there.

#### Objectives

This trial aims to explore the clinical value of clarithromycin-contained quadruple therapy based on faecal molecular antimicrobial susceptibility tests as the first-line treatment for *H. pylori* infection to improve the eradication rate.

#### Trial design and setting

This study is designed as a single-centred, single-blind, randomised trial. Group A will be treated with amoxicillin-based and clarithromycin-based BQT; group B and group C will be treated with amoxicillin-based and clarithromycin-based BQT or amoxicillin-based and furazolidone-based BQT according to the history of clarithromycin medication and the results of faecal antimicrobial susceptibility tests, respectively. This study will be conducted in the Department of Gastroenterology of Second Affiliated Hospital, School of Medicine, Zhejiang University (SAHZU) in China. The start of the recruitment is planned in March 2023 and should be completed within the following 1 year.
Participant selection

Inclusion criteria

Participants enrolled should meet the following criteria: (1) participants aged 18–65 with no history of eradication treatment; (2) diagnosed as *H. pylori* infection by one or more of the following methodologies: gastric biopsy using histochemical staining, tissue culture, the 14C-urea breath test (UBT), the 13C-UBT and/or faecal antigen; (3) were requested to undergo an endoscopy before the eradication treatment if they have alarm symptoms, a family history of gastric cancer or age over 40 years old without undergoing an endoscopy before and (4) voluntarily participated in the clinical trial and have signed the informed consent.

Exclusion criteria

Potential participants will be screened on the following exclusion criteria: (1) participants had a history of using antibiotics or bismuth within 4 weeks or acid inhibitor (including H2 receptor antagonist (H2RA), proton-pump inhibitor (PPI) or potassium-competitive acid blocker (P-CAB)) within 2 weeks before inclusion; (2) had an active peptic ulcer with complications such as haemorrhage, perforation or obstruction; (3) had a history of esophagectomy or gastrectomy; (4) had an allergy to any study drug; (5) were pregnant or breast feeding; (6) had a history of alcohol abuse or drug addiction; (7) existence of mental illness or (8) without self-judgement ability.

Sample size calculation

Based on previous studies, we assume the eradication rate of each group as 85%, 90% and 95%, respectively.7 11 22 28–30 We calculated that at least 855 participants (285 patients per treatment group) were required for recruitment for a two-sided α value of 0.05 and power of 90%, with a rate of loss to follow-up of 20%. The sample size was calculated using PASS software, V.11.0.10 (NCSS, LLC).

Recruitment, randomisation and blinding

Eligible participants will be recruited from patients diagnosed with *H. pylori* infection following the inclusion and exclusion criteria. They will be randomly assigned to three groups (group A, group B and group C) in a 1:1:1 ratio using a computer-generated algorithm for the trial treatment (figure 1). Before the treatment, the baseline characteristics of participants will be collected, including gender, sex, body mass index, family history, drug allergy history, medical history (such as basic diseases, tumour history and so on), contact information, medication history within 3 months, the history of clarithromycin medication and clinical symptoms. The blind method was applied to participants based on a comprehensive evaluation of the feasibility of the study.

Study protocol

Eight hundred and fifty-five participants were randomly divided into three groups, with 285 patients in each group, and received anti-*H. pylori* treatment as follows:

- Group A (amoxicillin- and clarithromycin-based BQT): 10 mg rabeprazole and 200 mg colloidal bismuth were orally taken 0.5 hours before breakfast and dinner, and 1 g amoxicillin and 500 mg clarithromycin were orally taken 0.5 hours after breakfast and dinner. The above medicines are required to be taken within 2 hours every time and continuously for 14 days. 6–8 weeks after the treatment is finished, the kit for 13C-UBT or 14C-UBT will be reviewed.
- Group B (clarithromycin medication history-based BQT): participants with clarithromycin medication history: 10 mg rabeprazole and 200 mg colloidal bismuth were orally taken 0.5 hours before breakfast and dinner, and 1 g amoxicillin and 100 mg furazolidone were orally taken 0.5 hours after breakfast and dinner. Participants without clarithromycin medication history: 10 mg rabeprazole and 200 mg colloidal bismuth were orally taken 0.5 hours before breakfast and dinner, and 1 g amoxicillin and 500 mg clarithromycin were orally taken 0.5 hours after breakfast and dinner. The above medicines are required to be taken within 2 hours every time and continuously for 14 days. 6–8 weeks after the treatment is finished, the kit for 13C-UBT or 14C-UBT will be reviewed.
- Group C (antimicrobial susceptibility test-based BQT): faecal molecular antimicrobial susceptibility tests using PCR kits detecting 23S ribosomal RNA point mutations (Jiangsu Cowin Biotechnology, Taizhou, Jiangsu, China) related to clarithromycin and levofloxacin will be performed before the treatment. DNA from stool samples will be extracted using auto nucleic acid extraction instrument CWE 9600 (Thermo Fisher Scientific, Waltham, Massachusetts). The mutations in 23S rRNA and gyrA genes will be amplified using real-time PCR using ABI 7500 (Thermo Fisher Scientific, Waltham Massachusetts). The treatment regimen will be chosen according to the results of the antimicrobial susceptibility tests. Clarithromycin-sensitive participants: 10 mg rabeprazole and 200 mg colloidal bismuth were orally taken 0.5 hours before breakfast and dinner, and 1 g amoxicillin and 500 mg clarithromycin were orally taken 0.5 hours after breakfast and dinner. Clarithromycin-resistant participants: 10 mg rabeprazole and 200 mg colloidal bismuth were orally taken 0.5 hours before breakfast and dinner, and 1 g amoxicillin and 100 mg furazolidone were orally taken 0.5 hours after breakfast and dinner. The above medicines are required to be taken within 2 hours every time and continuously for 14 days. 6–8 weeks after the treatment is finished, the kit for 13C-UBT or 14C-UBT will be reviewed.

During the trial, the investigators need to maintain close contact with the participants to dynamically master participants’ compliance and provide guidance to them.
Study endpoints

Primary outcomes
The primary end point of this trial is eradication rates in each group. The kit for the $^{13}$C-UBT or $^{14}$C-UBT will be reviewed 6–8 weeks after the eradication treatment, and a negative result (<4.0%, delta over baseline of $^{13}$C-UBT; <100, disintegrations per minute of $^{14}$C-UBT) indicated a successful eradication. Patients were not permitted to take PPIs or P-CABs 2 weeks before the UBT or antibiotics 4 weeks before the UBT. Eradication rate=number of negative cases/total cases×100%. Secondary outcomes
The secondary end points were the incidence of AEs and compliance.

Safety assessments
The investigator will maintain close contact with the participants during the trial, record all AEs mentioned above on the case report form and offer medical support if necessary.

Data management
A specific data collection group will be established at the Department of Gastroenterology of SAHZU before the study. Two specific investigators with rich experience in data collation and analysis are responsible for the data collection and double check. The original data will be recorded on case report forms and an Excel (V.2022) chart accordingly and cross checked by the head investigator. All management processes associated with data

Figure 1  Flowchart of the clinical study. Group A: rabeprazole, amoxicillin, clarithromycin and colloidal bismuth. Group B: with clarithromycin medication history: rabeprazole, amoxicillin, furazolidone and colloidal bismuth; without clarithromycin medication history: rabeprazole, amoxicillin, clarithromycin and colloidal bismuth. Group C: clarithromycin-resistant: rabeprazole, amoxicillin, furazolidone and colloidal bismuth; clarithromycin-sensitive: rabeprazole, amoxicillin, clarithromycin and colloidal bismuth. AEs, adverse events; BQT, bismuth-containing quadruple therapy; $^{13}$C UBT, $^{13}$C-urea breath test; $^{14}$C-UBT, $^{14}$C-urea breath test; GSRS, gastrointestinal symptom rating scale.
access and analysis will be supervised by the Ethics Committees of SAHZU.

**Statistical analysis**

Non-normally distributed continuous variables will be presented as median and categorical variables as absolute frequencies (proportions). Continuous variables will be compared using the non-parametric Kruskal-Wallis test. Categorical variables will be compared using the \( \chi^2 \) test. Statistical significance was defined as \( p < 0.05 \). Statistical analyses will be performed using SPSS (V.26.0; IBM SPSS Statistics, IBM Corporation, Armon, New York) and GraphPad PRISM software (V.9.0; GraphPad Software, San Diego, California).

**Patient and public involvement**

No patients or members of the public participated in the conception of our study. The adverse reactions of patients during the trial need timely feedback for safety considerations. On completion, the final results will be fed back to the patients in case report forms and the results of the study will be published in the appropriate journal.

**Ethics and dissemination**

This study was approved by the Ethics Committee of SAHZU (No. 20230103). Any amendment to the research protocol will be submitted for ethical approval. All participants or his/her authorised agent must provide written informed consent (online supplemental material 1) before participating in the practice. On completion, the results of the study will be published in the appropriate peer-reviewed journal.

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**Contributors**

JY, QD and YWu conceptualised the study. JY, QD, HW and YWu are responsible for monitoring the processes of the trial. Wliang, XL, DL, GL, YL and HW supervised the field study. YWu and JY developed the first draft of the manuscript. YWu, JY and HW developed the revision of the manuscript. All the authors contributed to the development of the study protocol and approved the final draft of the manuscript.

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**Competing interests**

None declared.

**Patient and public involvement**

Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

**Patient consent for publication**

Not applicable.

**Provenance and peer review**

Not commissioned; externally peer reviewed.

**Supplemental material**

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