Clinical Trial Protocol and Statistical Analysis Plan

Contents

Original Clinical Trial Protocol

Efficacy of Clarithromycin Susceptibility-based Tailored Versus Empiric Therapy for Helicobacter pylori First-line Treatment：a Multicenter Randomized Controlled Trial

Protocol version: 1.0

Approved Date: Jul 8, 2016

Funding Source: *Study On Helicobacter pylori Eradication Treatment and Infection Related Risk Factor* project funded by Clinical Research Center, Shanghai Jiao Tong University School of Medicine, DLY201608.

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Principal Investigator

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Protocol Abstract

Title	Efficacy of Clarithromycin Susceptibility-based Tailored Versus Empiric Therapy for Helicobacter pylori First-line Treatment： a Multicenter Randomized Controlled Trial
Trial registration number	ClinicalTrials.gov, NCT02935010
Objective	Comparing the efficacy, safety and compliance of PCR molecular method and genotypic clarithromycin susceptibility-based tailored therapy with empiric modified bismuth quadruple therapy in treating naïve patients with H. pylori infection
Condition	H. pylori infection naïve to treatment
Study Design	This study is a prospective, superiority-designed, multi-center, open-label, randomized controlled trial. Eligible patients will be randomly allocated to receive either clarithromycin susceptibility-based tailored therapy (tailored therapy) or empirical bismuth quadruple therapy (empirical therapy) in a 3:1 ratio. Six weeks after completion of therapy, H. pylori eradication will be assessed by ^{13}C-urea breath testing.
Sample size	500
Inclusion Criteria	1. Participants with non-ulcer functional dyspepsia or scarred peptic ulcer disease； 2. Ability and willingness to participate in the study and to sign and give informed consent； 3. confirmed H. pylori infection with positive results on both rapid urease testing and culture

Exclusion Criteria	1. Previous H. pylori eradication therapy 2. Less than 18 years old 3. With history of H. pylori infection treatment 4. With previous gastric surgery 5. Major systemic diseases 6. Pregnancy or lactation 7. Allergy to any of the study drugs 8. Administration of antibiotics, bismuth, antisecretory drugs in 8 weeks prior to inclusion
Arms and Interventions	**1. Empirical therapy** After review of clarithromycin-taking medical history, if have used clarithromycin, give esomeprazole 20mg bid, bismuth potassium citrate 600mg bid, amoxicillin 1000mg bid and metronidazole 400mg bid for 14 days; if have not used clarithromycin, give esomeprazole 20mg bid, bismuth potassium citrate 600mg bid, amoxicillin 1000mg bid and clarithromycin 500mg bid. **2. Tailored therapy** After molecular genetic assays for identification of resistance to clarithromycin of Helicobacter pylori from biopsy samples, if resistance to clarithromycin, give esomeprazole 20mg bid, bismuth potassium citrate 600mg bid, amoxicillin 1000mg bid and metronidazole 400mg bid for 14 days; if susceptible to clarithromycin, give esomeprazole 20mg bid, bismuth potassium citrate 600mg bid, amoxicillin 1000mg bid and clarithromycin 500mg bid.

Schedule	**1. Enrollment** Obtain written informed form Check eligibility criteria Collect baseline characteristics Undergo upper endoscopy with gastric biopsy and rapid urease testing Conduct bacterial culture and antibiotic susceptibility testing **2. Allocation** After the written informed consents would be obtained from eligible patients, the independent research assistant will open envelopes by randomization sequence and telephone study staff to give them each patient's treatment allocation. Eligible patients will be randomly allocated to receive either tailored therapy or empirical therapy in a 3:1 ratio. **3. treatment** Study treatment regimens were all given 14 days. During the treatment period, the subjects kept a diary to monitor compliance and symptoms. **4. Follow-up** After the end of treatment, adverse events and compliance was surveyed. Six weeks after completion of therapy, H. pylori eradication was assessed by ^{13}C-urea breath testing.
Outcome Measures	**Primary Outcome Measure:** 1. Helicobacter pylori eradication rate Six weeks after completion of therapy, H. pylori eradication success is

	defined as negative result from urea breath test (<4‰) **Secondary Outcome Measure** 1. Rate of adverse events 2. Compliance rate **Other Pre-specified Outcome Measures:** 1. H. pylori eradication rate of patients with clarithromycin resistance strains 2. H. pylori eradication rate of patients with clarithromycin sensitive strains 3. Sensitivity, specificity, positive predictive value and negative predictive value of molecular genetic assays in clarithromycin resistance identification 4. Medical cost per patient of tailored or empiric therapy 5. Ratio of medical cost to H. pylori eradication rate of each therapy 6. Ratio of incremental medical cost to incremental H. pylori eradication rate of tailored compared with empiric therapy

Protocol Schedule

Study Period	Enrollment	Allocation	Treatment	Follow-up	
Visit	1st	2nd		3rd	4th
Time point (day)	-14th	0	0-14	15th	56th
Eligibility screen	√				
Baseline characteristics	√				
Endoscopy with biopsy and RUT	√				
H. pylori culture and susceptibility testing	√				
PCR molecular method	√				
Written informed content	√				
Therapy Allocation		√			
Treatment			√		
Adverse events				√	
compliance assessment				√	
^{13}C-urea breath test					√
Medical cost					√

1. Background

Helicobacter pylori (*H. pylori*) resistance to antibiotics was main challenge to previous efficacy antibiotic regimens such as standard triple therapy(1). The prevalence of antibiotic resistance have been markedly increasing with time in most parts of the world. Concordantly, the antimicrobial eradication rate of H. pylori has been declining globally.(2) In China, clarithromycin, metronidazole and levofloxacin resistance show similar trends, and resistance rates have now reached 21□52% for clarithromycin, 61~95% for metronidazole, 20~54% for levofloxacin, and 21~37% for both clarithromycin and metronidazole.(3–5) Standard triple therapy had not been suitable for first-line therapy in China because the pooled eradication rate had declined to 74.5% as a meta-analysis showed.(6)

As a general rule, therapy for an infectious disease starts with identification of potentially useful antimicrobials and is largely based on the results of susceptibility testing. Systematic reviews or meta-analysis had proved susceptibility-based therapy was superior to 7–10 day triple therapies as first-line treatment.(7–9) Therefore, there is a critical need to antimicrobial susceptibility testing (AST) for individualized analysis of antibiotic resistance prior to definitive treatment.(2)

Now culture-based susceptibility testing for H pylori is rarely performed in standard practice. This may be due to several major limitations: they are time-consuming, costly, and invasive procedures, culture difficulty, and et al. In the age of molecular diagnostics, assays based on genetic material identification have become common as they enable acquiring rapid and accurate results at relatively low costs. The mechanism of resistance to clarithromycin in H. pylori is attributed to a decreased potency in binding of macrolides to the ribosome, caused by point mutations within the *rrl* gene in the peptidyl transferase- encoding region of the 23S rRNA gene. Three major point mutations in two positions, A2142C, A2142G, and A2143G, is the main cause of resistance to clarithromycin.(10)

So in this study, we will evaluate the efficacy, safety and compliance of PCR molecular method and genotypic clarithromycin susceptibility-based tailored therapy to treat naïve patients with *H. pylori* infection. The comparator is novel bismuth quadruple therapy which has been proved highly effective.(11)

2. Objective

2.1 The primary objective

To Comparing the efficacy of tailored therapy with empiric modified bismuth quadruple therapy in treating naïve patients with H. pylori infection.

2.2 The secondary objective

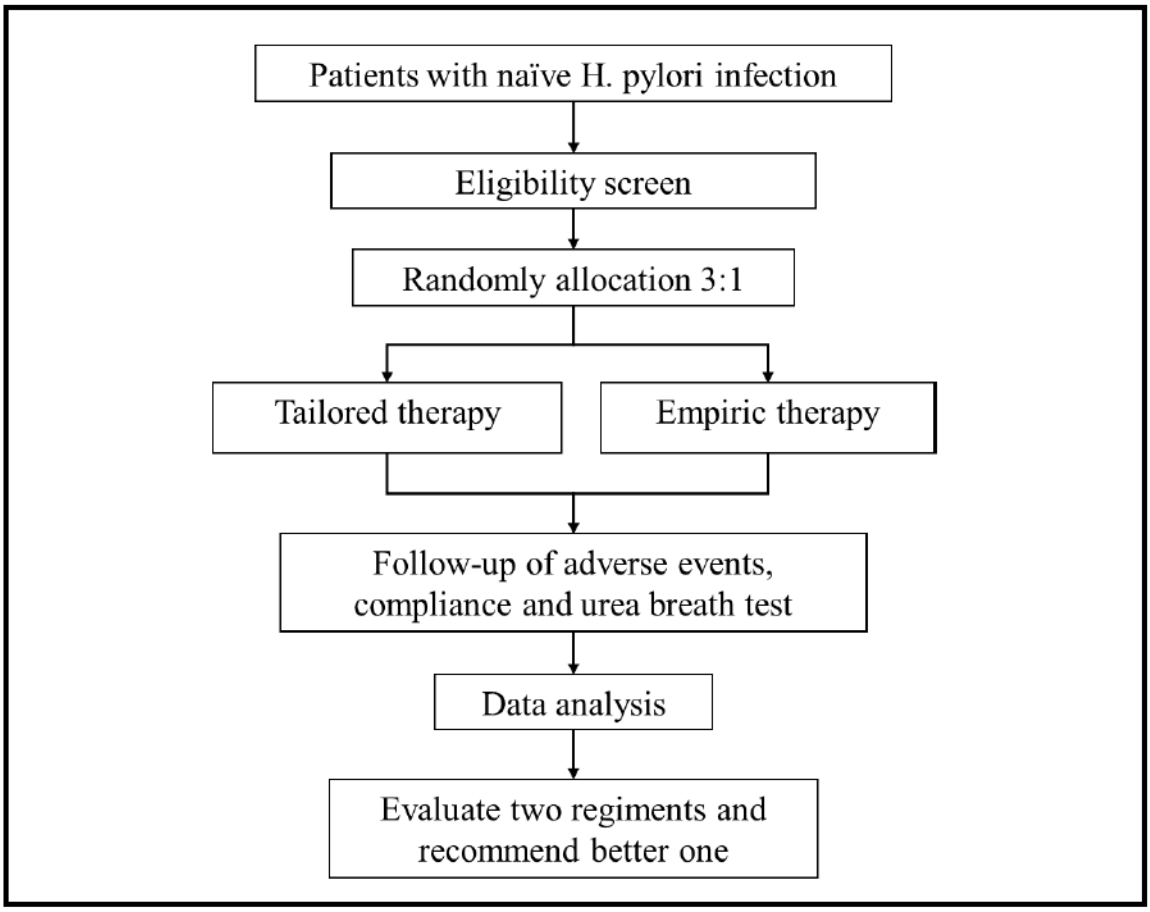
(1) To compare the safety and compliance of tailored therapy with empiric modified bismuth quadruple therapy;

(2) To evaluate the impact of clarithromycin resistance on eradication rates;

(3) To evaluate the diagnosis accuracy of molecular genetic assays in clarithromycin resistance identification

(4) To evaluate the cost-effectiveness of tailored therapy in treating naïve patients with H. pylori infection.

3. Follow Diagram



4. Study Design

This study is a prospective, superiority-designed, multi-center, open-label, randomized controlled trial conducted in five hospitals in Shanghai, China including Renji Hospital, Ruijin Hospital, Zhongshan Hospital, Shanghai general Hospital, and Shanghai Tenth People's Hospital.

5. Study Population

5.1 Inclusion criteria

(1) Participants with non-ulcer functional dyspepsia or scarred peptic ulcer disease;

(2) Ability and willingness to participate in the study and to sign and give informed consent;

(3) confirmed H. pylori infection with positive results on both rapid urease testing and culture

5.2 Exclusion criteria

(1) Previous H. pylori eradication therapy

(2) Less than 18 years old

(3) With history of H. pylori infection treatment

(4) With previous gastric surgery

(5) Major systemic diseases

(6) Pregnancy or lactation

(7) Allergy to any of the study drugs

(8) Administration of antibiotics, bismuth, antisecretory drugs in 8 weeks prior to inclusion

5.3 Drop out standard

Subjects who sign the informed consent and do not complete the prescribed observation period regardless of when and where to exit, are called drop out.

(1)Patients found in the study course who do not meet the inclusion criteria or do follow the exclusion standards.

(2)Patients who get other irrelevant diseases that could affect the assessment on the efficiency and safety of the drug during the test.

(3)Patients who receive any drug that influences the assessment on the drug.

(4)Patients who do not strictly take standard medication because of any reason (e.g. severe side effects).

5.4 Termination standard

Test termination refers to that clinical trials stop before the end, for the purpose to protect the rights and interests of subjects and to ensure the test quality.

(1)Sever safety issues occur during the trials;

(2)Intervention drug was proved poor or no efficacy;

(3)Serious medical errors in protocol that makes estimation impossible;

(4) Administration reasons;

(5)Withdraw by State food and drug administration.

6. Allocation and Treatment

6.1 Randomization and blinding

Eligible patients will be randomly allocated to receive either susceptibility-based tailored therapy (tailored therapy) or empirical bismuth quadruple therapy (empirical therapy) in a 3:1 ratio. An independent statistician at Shanghai Jiao Tong University College of Basic Medical Sciences generates the computerized random number sequence and used a permuted block randomization with a block size of eight. All investigators is masked to the randomization sequence. Allocation is concealed in an opaque envelope until the intervention was assigned. Envelopes will be kept at Renji Hospital. After the written informed consents would be obtained from eligible patients, the independent research assistant will open envelopes by randomization sequence and telephone study staff to give them each patient's treatment allocation. This study is an open-labeled trial and patients are not blinded. The technicians, who perform the H. pylori tests (rapid urease test, culture, antimicrobial susceptibility testing, and urea breath test) or fill in the questionnaires, will be blinded to treatment allocation.

6.2 Intervention

(1) Empirical therapy

After review of clarithromycin-taking medical history, if have used clarithromycin, give esomeprazole 20mg bid, bismuth potassium citrate 600mg bid, amoxicillin 1000mg bid and metronidazole 400mg bid for 14 days; if have not used clarithromycin, give

esomeprazole 20mg bid, bismuth potassium citrate 600mg bid, amoxicillin 1000mg bid and clarithromycin 500mg bid for 14 days.

(2) Tailored therapy

After molecular genetic assays for identification of resistance to clarithromycin of Helicobacter pylori from biopsy samples, if resistance to clarithromycin, give esomeprazole 20mg bid, bismuth potassium citrate 600mg bid, amoxicillin 1000mg bid and metronidazole 400mg bid for 14 days; if susceptible to clarithromycin, give esomeprazole 20mg bid, bismuth potassium citrate 600mg bid, amoxicillin 1000mg bid and clarithromycin 500mg bid for 14 days.

6.3 Drugs

Esomeprazole, 20mg/tablet, AstraZeneca AB, Sodertalje, Sweden.

Bismuth potassium citrate, 300mg/capsule (110mg elemental bismuth), Dawnrays Pharmaceutical Limited, Suzhou, Jiangsu, China.

Amoxicillin, 250mg/capsule, Ruiyang Pharmaceutical Co. Ltd., Shandong, China.

Clarithromycin, 250mg/tablet, Shanghai Abbott Laboratories Co. Ltd., Shanghai, China.

Metronidazole, 200mg/tablet, Shanghai Xinyi Wanxiang Pharmaceutical Industry Company Limited, Shanghai, China.

7. *H. pylori* real-time PCR and Culture Based Antibiotic Susceptibility Testing

Tissue samples will be obtained from gastroscopic biopsy.

DNAs will be isolated from biopsy specimens and mutation of clarithromycin resistance will be identified by standard molecular (real-time PCR) instrument systems and The *Helicobacter pylori* Analyte Specific Reagents (BIOLINE USA Inc., MA,

USA) according to the manufacturer's guidelines.

The biopsy specimens will be cultured and maintained on brain heart infusion agar medium (OXOID, Basingstoke, UK) containing 5% defibrinated sheep blood under microaerophilic conditions (85% N2, 10% CO2, 5% O2) at 37℃. All isolates will be stored in brain heart infusion broth (Difco Laboratory, Detroit, MI, USA) supplemented with 30% glycerol at -80℃. Clinical isolates will be also identified as H. pylori using positive tests for urease, oxidase, catalase and Gram staining.

Minimal inhibitory concentrations (MIC) of clarithromycin and metronidazole will be determined by the two-fold agar dilution method. H. pylori is suspended in saline and measured using a spectrophotometer. The bacterial suspensions (108 colony forming units per milliliter) are then plated with an inoculator (Sakuma Seisaku, Tokyo, Japan) onto agar plates containing various concentrations of above antibiotics. After three days of microaerophilic incubation, MIC is defined as the lowest drug concentration that prevented visible growth of bacteria. ATCC43504 is used as the quality control. Clarithromycin >2 μg/mL, and metronidazole >8 μg/mL is defined as resistance breakpoints.

8. Safety evaluation

From the beginning of that patients signed the informed consent and are selected for trial to one month after the end of treatment, any adverse medical events, regardless of whether a causal relationship with the study medication, will be judged to be Adverse Event (AE).

8.1 AE's degree

Mild: patients are easier to accept without induced questions, or patients have only mild discomfort which does not affect their daily lives and there is no need for clinical treatment.

Moderate: patients actively describe the symptoms that affect the life, but they can

tolerate, which doesn't need clinical treatment.

Severe: patients have objective manifestations, which significantly affect the life, but patients can or can't bear, which needs clinical treatment.

8.2 Records and follow-up of AE

During the study, AE will be accurately recorded, including the time of occurrence, severity, duration, the measures taken and the outcome. Researchers will follow all the AE until the symptoms of patients disappeared or condition become stable. SAE should be tracked until a proper solution is found even though the study is over.

8.3 Serious adverse event (SAE)

(1)The judgments of SAE:

□ Death, Life-threatening,

□ Leading to hospitalization or prolong hospitalization time,

□ Permanent or severe disability,

□ Congenital Anomaly/Birth Defect.

(2)SAE report system:

Principal investigator and the hospital ethics committee should be reported and SAE report form should be filled in within 24 hours by phone no matter whether any kinds of SAE are related with the drug in 30 days after the treatment. And the form should be reported to national drug administration in time by principal investigator. SAE should be promptly handled, closely tracked until it is properly solved.

Contact method:

1) Contact persons: Hong Lu 86-13611958022

2) SFDA Safety Supervision

9. Outcome Measures

9.1 Primary Outcome Measure:

1. Helicobacter pylori eradication rate

Six weeks after completion of therapy, H. pylori eradication success is defined as negative result from urea breath test (<4‰). If patients would be lost to follow-up urea breath testing, they will be scored as treatment failures in the primary ITT analysis. The eradication rate is defined as the number of patients who successful eradicate *H. pylori* divided by the total number of population for analysis.

9.2 Secondary Outcome Measure

(1) Rate of adverse events

During the 14-day treatment period, the subjects will keep a diary to score any possible side effects or discomforts. The subjects will be asked to grade the severity of adverse events according to their influence on daily activities, experienced as “mild” (transient and well tolerated), “moderate” (causing discomfort and partially interfering with daily activities), or “severe” (causing considerable interference with daily activities). The side effect score recorded is based on the most severe event. Rate of adverse events is defined as the number of patients with any adverse event divided by the total number of population for analysis.

(2) Compliance rate

Compliance was defined as either poor when they had taken less than 80% of the total medication or good when they had taken more than 80% medication. Compliance rate is defined as the number of patients with good compliance divided by the total number of population for analysis.

9.3 Other Pre-specified Outcome Measures:

(1) H. pylori eradication rate of patients with clarithromycin resistance strains

(2) H. pylori eradication rate of patients with clarithromycin sensitive strains

(3) Sensitivity, specificity, positive predictive value and negative predictive value of molecular genetic assays in clarithromycin resistance identification

(4) Medical cost per patient of tailored or empiric therapy

(5) Ratio of medical cost to H. pylori eradication rate of each therapy

(6) Ratio of incremental medical cost to incremental H. pylori eradication rate of tailored compared with empiric therapy

10. Statistical Methods

10.1 Sample size

Assuming 95.7% eradication rate of tailored therapy in our trial, 88.8% eradication rate of empirical therapy, a superiority margin of >0, a power of 80%, an alpha of 0.05 (one-sided), a ratio of 3:1, at least 340 subjects in susceptibility-based therapy and 114 subjects in empirical therapy will be required to show superiority of susceptibility-based therapy over empirical therapy on the basis of H. pylori eradication rate. Taking into consideration of 10% lost to follow-up, at least 500 participants (374 for tailored therapy and 126 for empiric therapy) is expected to be recruited for the study.

10.2 Statistical Analysis Data

Intention--to-treat (ITT) Analysis population: According to the Intention-to-treat principle: All randomized patients will be included in the ITT analysis. Patients who did not return for a follow-up 13C-UBT will be recorded as treatment failures.

Per-protocol (PP) analysis population: All individuals who violated the study protocol, such as patients not taking at least 80% of treatment drugs, or with unknown post-treatment H. pylori status will be excluded from the PP analysis.

Safety analysis population: The patients with missing safety data in ITT population will excluded from the safety analysis.

10.3 Statistical methods

Comparative superiority of the two groups was assessed through hypothesis testing (one-sided Z test) and derivation of a two-sided 95% confidence interval (CI) of difference based on H. pylori eradication rate. If p-value of the testing less than 0.025 and the lower bound of the 95% CI greater than zero, superiority of susceptibility-based therapy over empirical therapy could be concluded.

Between-group differences were evaluated using Student's t-test for continuous variables and Pearson's $\chi 2$/corrected $\chi 2$ or Fisher's exact test for categorical variables, as appropriate. All p-values were two-sided except the testing of superiority, and were considered statistically significant if p-value less than 0.05.

Subgroup analyses of eradication efficacy were performed based on the results of antibiotic susceptibility test and compliance assessment.

11 Ethical principles

The study is registered in ClinicalTrials.gov. The study protocol is approved by the Ethics Committee at all institutions, and the study will be performed according to good clinical practice and the Declaration of Helsinki.

Before each patient is selected into the study, the physician of the study have the responsibility to tell patients or their designated agents the purpose, process, completion and comprehensively introduce possible adverse reactions, risks to bear, possible benefits and other information all-round and detailed, making the patients know their rights. We are ordered to inform the patients of having the right to decide whether to participate in the study, as well as the right to withdraw the study at any time without any discrimination. The patients or their legal representatives should sign the informed consent after carefully reading and fully understanding, and retain the signature page of the copy.

12 References

1. Malfertheiner P, Megraud F, O'Morain CA, et al. Management of Helicobacter pylori infection—the Maastricht IV/Florence Consensus Report. Gut 2012;61:646–64.

2. Thung I, Aramin H, Vavinskaya V, Gupta S, Park JY, Crowe SE, et al. Review article: the global emergence of Helicobacter pylori antibiotic resistance. Aliment Pharmacol Ther. 2016 Feb 1;43(4):514–33.

3. Su P, Li Y, Li H, Zhang J, Lin L, Wang Q, et al. Antibiotic Resistance of Helicobacter Pylori Isolated in the Southeast Coastal Region of China. Helicobacter. 2013 Aug 1;18(4):274–9.

4. Bai P, Zhou LY, Xiao XM, Luo Y, Ding Y. Susceptibility of Helicobacter pylori to antibiotics in Chinese patients. J Dig Dis. 2015 Aug;16(8):464–70.

5. Zhang Y-X, Zhou L-Y, Song Z-Q, Zhang J-Z, He L-H, Ding Y. Primary antibiotic resistance of Helicobacter pylori strains isolated from patients with dyspeptic symptoms in Beijing: A prospective serial study. World J Gastroenterol WJG. 2015 Mar 7;21(9):2786–92.

6. Wang B, Lv Z-F, Wang Y-H, Wang H, Liu X-Q, Xie Y, et al. Standard triple therapy for Helicobacter pylori infection in China: A meta-analysis. World J Gastroenterol. 2014 Oct 28;20(40):14973–85.

7. Wenzhen Y, Yumin L, Quanlin G, Kehu Y, Lei J, Donghai W, et al. Is Antimicrobial Susceptibility Testing Necessary Before First-line Treatment for Helicobacter pylori Infection? -Meta-analysis of Randomized Controlled Trials-. Intern Med. 2010;49(12):1103–9.

8. López-Góngora S, Puig I, Calvet X, Villoria A, Baylina M, Muñoz N, et al. Systematic review and meta-analysis: susceptibility-guided versus empirical antibiotic treatment for Helicobacter pylori infection. J Antimicrob Chemother. 2015

Sep;70(9):2447–55.

9. Chen H, Dang Y, Zhou X, Liu B, Liu S, Zhang G. Tailored Therapy Versus Empiric Chosen Treatment for Helicobacter pylori Eradication: A Meta-Analysis. Medicine (Baltimore). 2016 Feb;95(7):e2750.

10. De Francesco V, Zullo A, Ierardi E, et al. Phenotypic and genotypic Helicobacter pylori clarithromycin resistance and therapeutic outcome: benefits and limits. J Antimicrob Chemother. 2010;65:327-332.

11. Zhang W, Chen Q, Liang X, Liu W, Xiao S, Graham DY, et al. Bismuth, lansoprazole, amoxicillin and metronidazole or clarithromycin as first-line Helicobacter pylori therapy. Gut. 2015 Nov;64(11):1715–20.

Signature Page

Protocol Title:

Efficacy of Antibiotic Susceptibility-based Tailored Versus Empiric Therapy for Helicobacter pylori First-line Treatment：a Multicenter Randomized Controlled Trial

After reviewing the protocol, each investigator and statistician signed this page as an attachment.

Investigator:

I will earnestly fulfill my duties as an investigator in accordance with the *Chinese Good Clinical Practice* (GCP).

This study will be conducted in accordance with the principles of morality, ethics and science laid down in the Helsinki Declaration and the Chinese GCP. I agree to carry out this clinical trial in accordance with this protocol.

I will be responsible for making timely and appropriate medical decisions for adverse events that occur in subjects during the study period. I know the requirements for correctly reporting serious adverse events and I will record and report these events.

I guarantee that the data will be accurately, completely, timely, and legally filled in the Case Report Form. I will accept the inspections to ensure the quality of the study.

I agree that the findings of the study are published.

Principal Investigator: Hong Lu

Institution: Renji Hospital, School of Medicine, Shanghai Jiao Tong University,

Shanghai, China.

Signature: _____

Co-investigator: Yunwei Sun

Institution: Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

Signature: _____

Co-investigator: Hong Gao

Institution: Zhongshan Hospital, Fudan University, Shanghai, China

Signature: _____

Co-investigator: Yan Zhan

Institution: Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai, China.

Signature: _____

Co-investigator: Gang Xu

Institution: Department of Gastroenterology and Hepatology, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

Signature: _____

Statistical unit

We will conduct statistical analysis based on the Chinese "guideline of Biostatistics for Clinical Trials of Chemical Drugs and Biological Products" and related regulations.

Statistician: Yanyan Song

Institution: Institute of Medical Sciences, Shanghai Jiao Tong University School of Medicine, Shanghai, China

Signature: _____

Final Clinical Trial Protocol

Efficacy of Antibiotic Susceptibility-based Tailored Versus Empiric Therapy for Helicobacter pylori First-line Treatment：a Multicenter Randomized Controlled Trial

Protocol version: 1.1

Revised Date: Jan 10, 2017

Funding Source: *Study On Helicobacter Pylori Eradication Treatment and Related Risk Factors of Infection* project funded by Clinical Research Center, Shanghai Jiao Tong University School of Medicine, DLY201608.

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Statistician

Yanyan Song, Department of Biostatistics, Institute of Medical Sciences, Shanghai Jiao Tong University School of Medicine, Shanghai, China

Protocol Abstract

Title	Efficacy of Antibiotic Susceptibility-based Tailored Versus Empiric Therapy for Helicobacter pylori First-line Treatment：a Multicenter Randomized Controlled Trial
Trial registration number	ClinicalTrials.gov, NCT02935010
Objective	Comparing the efficacy, safety and compliance of pretreatment culture and susceptibility-based tailored therapy with empiric modified bismuth quadruple therapy in treating naïve patients with H. pylori infection
Condition	H. pylori infection naïve to treatment
Study Design	This study is a prospective, superiority-designed, multi-center, open-label, randomized controlled trial. Eligible patients will be randomly allocated to receive either susceptibility-based tailored therapy (tailored therapy) or empirical bismuth quadruple therapy (empirical therapy) in a 3:1 ratio. Six weeks after completion of therapy, H. pylori eradication will be assessed by ^{13}C-urea breath testing.
Sample size	382
Inclusion Criteria	1. Participants with non-ulcer functional dyspepsia or scarred peptic ulcer disease； 2. Ability and willingness to participate in the study and to sign and give informed consent； 3. confirmed H. pylori infection with positive results on both rapid urease testing and culture

Exclusion Criteria	1. Previous H. pylori eradication therapy 2. Less than 18 years old 3. With history of H. pylori infection treatment 4. With previous gastric surgery 5. Major systemic diseases 6. Pregnancy or lactation 7. Allergy to any of the study drugs 8. Administration of antibiotics, bismuth, antisecretory drugs in 8 weeks prior to inclusion
Arms and Interventions	**1. Empirical therapy** Esomeprazole 20 mg, bismuth potassium citrate 600 mg taken twice a day, amoxicillin 1 g, and metronidazole 500 mg taken three times a day for 14 days. **2. Tailored therapy** Tailored therapy is based on antimicrobial susceptibility of clarithromycin, metronidazole and levofloxacin. according to antibiotic resistance pattern included esomeprazole 20mg bid, amoxicillin 1g bid, with a third drug (clarithromycin 500mg bid, metronidazole 400mg bid, or levofloxacin 500mg qd) for susceptible strains, or with bismuth 220mg bid plus metronidazole 400mg qid for triple-resistant strains. Study treatment regimens were all given 14 days.
Schedule	**1. Enrollment** Obtain written informed form

	Check eligibility criteria Collect baseline characteristics Undergo upper endoscopy with gastric biopsy and rapid urease testing Conduct bacterial culture and antibiotic susceptibility testing **2. Allocation** After the written informed consents would be obtained from eligible patients, the independent research assistant will open envelopes by randomization sequence and telephone study staff to give them each patient's treatment allocation. Eligible patients will be randomly allocated to receive either tailored therapy or empirical therapy in a 3:1 ratio. **3. treatment** Study treatment regimens were all given 14 days. During the treatment period, the subjects kept a diary to monitor compliance and symptoms. **4. Follow-up** After the end of treatment, adverse events and compliance was surveyed. Six weeks after completion of therapy, H. pylori eradication was assessed by ^{13}C-urea breath testing.
Outcome Measures	**Primary Outcome Measure:** 1. Helicobacter pylori eradication rate Six weeks after completion of therapy, H. pylori eradication success is defined as negative result from urea breath test (<4‰) **Secondary Outcome Measure**

	1. Rate of adverse events 2. Compliance rate **Other Pre-specified Outcome Measures:** 1. Medical cost per patient of tailored or empiric therapy 2. Ratio of medical cost to H. pylori eradication rate of each therapy 3. Ratio of incremental medical cost to incremental H. pylori eradication rate of tailored compared with empiric therapy

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Visit	1st	2nd		3rd	4th
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Eligibility screen	√				
Baseline characteristics	√				
Endoscopy with biopsy and RUT	√				
H. pylori culture and susceptibility testing	√				
Written informed content	√				
Therapy Allocation		√			
Treatment			√		
Adverse events				√	
compliance assessment				√	
^{13}C-urea breath test					√
Medical cost					√

1. Background

Helicobacter pylori (*H. pylori*) resistance to antibiotics was main challenge to previous efficacy antibiotic regimens such as standard triple therapy(1). The prevalence of antibiotic resistance have been markedly increasing with time in most parts of the world. Concordantly, the antimicrobial eradication rate of H. pylori has been declining globally.(2) In China, clarithromycin, metronidazole and levofloxacin resistance show similar trends, and resistance rates have now reached 21□52% for clarithromycin, 61~95% for metronidazole, 20~54% for levofloxacin, and 21~37% for both clarithromycin and metronidazole.(3–5) Standard triple therapy had not been suitable for first-line therapy in China because the pooled eradication rate had declined to 74.5% as a meta-analysis showed.(6)

As a general rule, therapy for an infectious disease starts with identification of potentially useful antimicrobials and is largely based on the results of susceptibility testing. Systematic reviews or meta-analysis had proved susceptibility-based therapy was superior to 7–10 day triple therapies as first-line treatment.(7–9) Therefore, there is a critical need to antimicrobial susceptibility testing (AST) for individualized analysis of antibiotic resistance prior to definitive treatment.(2)

Now culture-based susceptibility testing for H pylori is rarely performed in standard practice. This may be due to several major limitations: they are time-consuming, costly, and invasive procedures, culture difficulty, and et al. So empirical therapy would be needed for most patients over the world. In the settings with high antibiotic resistance, only bismuth quadruple therapy (BQT) is the recommended first-line treatment, and any non-BQT therapy (triple, concomitant, hybrid, or sequential) would not be more effective option.(1) Our previous study showed that substitution of amoxicillin for tetracycline in a 14-day BQT containing 1,600mg/d of metronidazole eradicated 96.4%(54/56) of metronidazole susceptible strains and 93.3%(42/45) of metronidazole resistant strains when used as first-line *H. pylori* therapy.(10). Fewer studies had

compared the effect of susceptibility-based therapy versus BQT for first-line H. pylori eradication, except one.(11)

In this study, we will evaluate the efficacy, safety and compliance of pretreatment culture and susceptibility-based tailored therapy to treat naïve patients with *H. pylori* infection. Susceptibility-based therapy will use Pref. Graham DY et al's recommendation, in which proton pump inhibitors, amoxicillin, and third susceptible drug triple therapies will be given after testing the susceptibility to clarithromycin, metronidazole and levofloxacin, or bismuth quadruple therapy was given if no susceptible antibiotics.(12) The comparator is novel bismuth quadruple therapy in which tetracycline was replaced by amoxicillin, without reference to antimicrobial susceptibility.

2. Objective

2.1 The primary objective

To Comparing the efficacy of tailored therapy with empiric modified bismuth quadruple therapy in treating naïve patients with H. pylori infection.

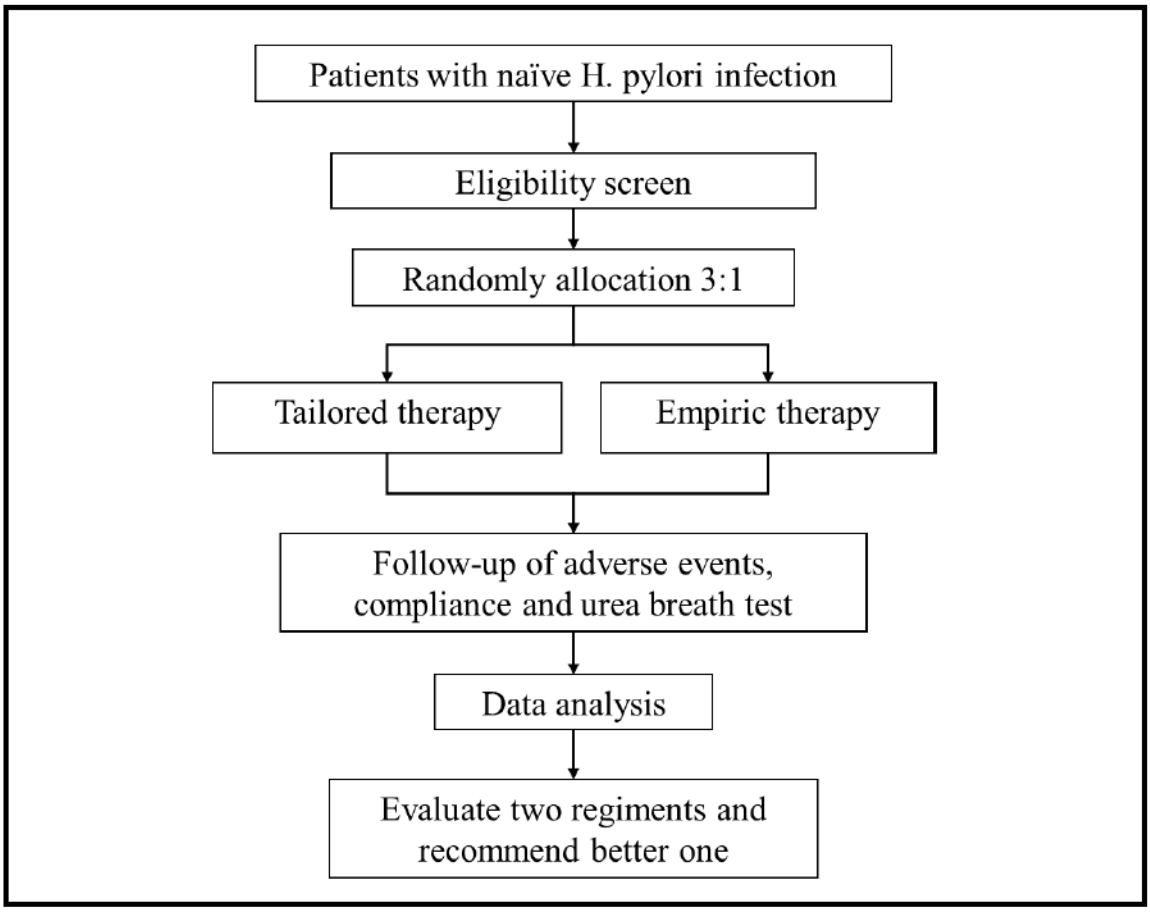
2.2 The secondary objective

(1) To compare the safety and compliance of tailored therapy with empiric modified bismuth quadruple therapy;

(2) To evaluate the impact of antibiotic resistance on eradication rates;

(3) To evaluate the cost-effectiveness of susceptibility-based tailored therapy in treating naïve patients with H. pylori infection.

3. Follow Diagram



4. Study Design

This study is a prospective, superiority-designed, multi-center, open-label, randomized controlled trial conducted in five hospitals in Shanghai, China including Renji Hospital, Ruijin Hospital, Zhongshan Hospital, Shanghai general Hospital, and Shanghai Tenth People's Hospital.

5. Study population

5.1 Inclusion criteria

(1) Participants with non-ulcer functional dyspepsia or scarred peptic ulcer disease;

(2) Ability and willingness to participate in the study and to sign and give informed consent;

(3) confirmed H. pylori infection with positive results on both rapid urease testing and culture

5.2 Exclusion criteria

(1) Previous H. pylori eradication therapy

(2) Less than 18 years old

(3) With history of H. pylori infection treatment

(4) With previous gastric surgery

(5) Major systemic diseases

(6) Pregnancy or lactation

(7) Allergy to any of the study drugs

(8) Administration of antibiotics, bismuth, antisecretory drugs in 8 weeks prior to inclusion

5.3 Drop out standard

Subjects who sign the informed consent and do not complete the prescribed observation period regardless of when and where to exit, are called drop out.

(1)Patients found in the study course who do not meet the inclusion criteria or do follow the exclusion standards.

(2)Patients who get other irrelevant diseases that could affect the assessment on the efficiency and safety of the drug during the test.

(3)Patients who receive any drug that influences the assessment on the drug.

(4)Patients who do not strictly take standard medication because of any reason (e.g. severe side effects).

5.4 Termination standard

Test termination refers to that clinical trials stop before the end, for the purpose to protect the rights and interests of subjects and to ensure the test quality.

(1)Sever safety issues occur during the trials;

(2)Intervention drug was proved poor or no efficacy;

(3)Serious medical errors in protocol that makes estimation impossible;

(4) Administration reasons;

(5)Withdraw by State food and drug administration.

6. Allocation and Treatment

6.1 Randomization and blinding

Eligible patients will be randomly allocated to receive either susceptibility-based tailored therapy (tailored therapy) or empirical bismuth quadruple therapy (empirical therapy) in a 3:1 ratio. An independent statistician at Shanghai Jiao Tong University College of Basic Medical Sciences generates the computerized random number sequence and used a permuted block randomization with a block size of eight. All investigators is masked to the randomization sequence. Allocation is concealed in an opaque envelope until the intervention was assigned. Envelopes will be kept at Renji Hospital. After the written informed consents would be obtained from eligible patients, the independent research assistant will open envelopes by randomization sequence and telephone study staff to give them each patient's treatment allocation. This study is an open-labeled trial and patients are not blinded. The technicians, who perform the H. pylori tests (rapid urease test, culture, antimicrobial susceptibility testing, and urea breath test) or fill in the questionnaires, will be blinded to treatment allocation.

6.2 Intervention

(1) Empirical therapy

Esomeprazole 20 mg, bismuth potassium citrate 600 mg taken twice a day, amoxicillin 1 g, and metronidazole 500 mg taken three times a day for 14 days.

(2) Tailored therapy

Tailored therapy is based on antimicrobial susceptibility of clarithromycin, metronidazole and levofloxacin. a) If the H. pylori strain of one patient was sensitive to clarithromycin, clarithromycin triple therapy (EAC) will be given: esomeprazole 20 mg, amoxicillin 1 g, and clarithromycin 500 mg taken twice a day; b) If the strain was resistant to clarithromycin but sensitive to metronidazole, metronidazole triple therapy (EAM) will be given: esomeprazole 20 mg, amoxicillin 1 g, and metronidazole 500 mg taken twice a day. c) If the strain was resistant to both clarithromycin and metronidazole but sensitive to levofloxacin, levofloxacin triple therapy (EAL) will be given: esomeprazole 20 mg, amoxicillin 1 g taken twice a day, and levofloxacin 500 mg taken once a day. d) If the strain was resistant to all three antibiotics, bismuth quadruple therapy (EBA_2M_4) will be given: esomeprazole 20 mg, bismuth potassium citrate 600 mg, amoxicillin 1 g taken twice a day, and metronidazole 500 mg taken four times a day. Study treatment regimens will be all given 14 days.

6.3 Drugs

Esomeprazole, 20mg/tablet, AstraZeneca AB, Sodertalje, Sweden.

Bismuth potassium citrate, 300mg/capsule (110mg elemental bismuth), Dawnrays Pharmaceutical Limited, Suzhou, Jiangsu, China.

Amoxicillin, 250mg/capsule, Ruiyang Pharmaceutical Co. Ltd., Shandong, China.

Clarithromycin, 250mg/tablet, Shanghai Abbott Laboratories Co. Ltd., Shanghai, China.

Metronidazole, 200mg/tablet, Shanghai Xinyi Wanxiang Pharmaceutical Industry Company Limited, Shanghai, China.

Levofloxacin, 500mg/tablet, Daiichi Sankyo Pharmaceutical Beijing Co., Ltd., Beijing, China.

7. *H. pylori* Culture and Antibiotic Susceptibility Testing

Tissue samples will be obtained from gastroscopic biopsy. The biopsy specimens will be cultured and maintained on brain heart infusion agar medium (OXOID, Basingstoke, UK) containing 5% defibrinated sheep blood under microaerophilic conditions (85% N2, 10% CO2, 5% O2) at 37℃. All isolates will be stored in brain heart infusion broth (Difco Laboratory, Detroit, MI, USA) supplemented with 30% glycerol at -80℃. Clinical isolates will be also identified as H. pylori using positive tests for urease, oxidase, catalase and Gram staining.

Minimal inhibitory concentrations (MIC) of clarithromycin, metronidazole, and levofloxacin will be determined by the two-fold agar dilution method. H. pylori is suspended in saline and measured using a spectrophotometer. The bacterial suspensions (108 colony forming units per milliliter) are then plated with an inoculator (Sakuma Seisaku, Tokyo, Japan) onto agar plates containing various concentrations of above antibiotics. After three days of microaerophilic incubation, MIC is defined as the lowest drug concentration that prevented visible growth of bacteria. ATCC43504 is used as the quality control. Clarithromycin >2 μg/mL, metronidazole >8 μg/mL, levofloxacin >2 μg/mL is defined as resistance breakpoints.

8. Safety evaluation

From the beginning of that patients signed the informed consent and are selected for trial to one month after the end of treatment, any adverse medical events, regardless of whether a causal relationship with the study medication, will be judged to be Adverse Event (AE).

8.1 AE's degree

Mild: patients are easier to accept without induced questions, or patients have only mild discomfort which does not affect their daily lives and there is no need for clinical treatment.

Moderate: patients actively describe the symptoms that affect the life, but they can tolerate, which doesn't need clinical treatment.

Severe: patients have objective manifestations, which significantly affect the life, but patients can or can't bear, which needs clinical treatment.

8.2 Records and follow-up of AE

During the study, AE will be accurately recorded, including the time of occurrence, severity, duration, the measures taken and the outcome. Researchers will follow all the AE until the symptoms of patients disappeared or condition become stable. SAE should be tracked until a proper solution is found even though the study is over.

8.3 Serious adverse event (SAE)

(1)The judgments of SAE:

□ Death, Life-threatening,

□ Leading to hospitalization or prolong hospitalization time,

□ Permanent or severe disability,

□ Congenital Anomaly/Birth Defect.

(2)SAE report system:

Principal investigator and the hospital ethics committee should be reported and SAE report form should be filled in within 24 hours by phone no matter whether any kinds of SAE are related with the drug in 30 days after the treatment. And the form should be reported to national drug administration in time by principal investigator. SAE should be promptly handled, closely tracked until it is properly solved.

Contact method:

1) Contact persons: Hong Lu 86-13611958022

2) SFDA Safety Supervision

9. Outcome Measures

9.1 Primary Outcome Measure:

1. Helicobacter pylori eradication rate

Six weeks after completion of therapy, H. pylori eradication success is defined as negative result from urea breath test (<4‰). If patients would be lost to follow-up urea breath testing, they will be scored as treatment failures in the ITT analysis. The eradication rate is defined as the number of patients who successful eradicate *H. pylori* divided by the total number of population for analysis.

9.2 Secondary Outcome Measure

(1) Rate of adverse events

During the 14-day treatment period, the subjects will keep a diary to score any possible side effects or discomforts. The subjects will be asked to grade the severity of adverse events according to their influence on daily activities, experienced as “mild” (transient and well tolerated), “moderate” (causing discomfort and partially interfering with daily activities), or “severe” (causing considerable interference with daily activities). The side effect score recorded is based on the most severe event. Rate of adverse events is defined as the number of patients with any adverse event divided by the total number of population for analysis.

(2) Compliance rate

Compliance was defined as either poor when they had taken less than 80% of the total medication or good when they had taken more than 80% medication. Compliance rate is defined as the number of patients with good compliance divided by the total number of population for analysis.

9.3 Other Pre-specified Outcome Measures:

(1) Medical cost per patient of tailored or empiric therapy

Medical cost of one patient is overall cost including visit, endoscopy, H. pylori culture, susceptibility testing, eradication treatment, and adverse event treatment.

(2) Ratio of medical cost to H. pylori eradication rate of each therapy

(3)Ratio of incremental medical cost to incremental H. pylori eradication rate of tailored compared with empiric therapy

10. Statistical Methods

10.1 Sample size

Assuming 96.5% eradication rate of tailored therapy in our trial, 88.9% eradication rate of empirical therapy, a superiority margin of >0, a power of 80%, an alpha of 0.05 (one-sided), a ratio of 3:1, at least 260 subjects in susceptibility-based therapy and 87 subjects in empirical therapy will be required to show superiority of susceptibility-based therapy over empirical therapy on the basis of H. pylori eradication rate. Taking into consideration of 10% lost to follow-up, at least 382 participants (286 for tailored therapy and 96 for empiric therapy) is expected to be recruited for the study.

10.2 Statistical Analysis Data

Intention--to-treat (ITT) Analysis population: According to the Intention-to-treat principle: All randomized patients will be included in the ITT analysis. Patients who did not return for a follow-up 13C-UBT will be recorded as treatment failures.

Per-protocol (PP) analysis population: All individuals who violated the study protocol, such as patients not taking at least 80% of treatment drugs, or with unknown post-treatment H. pylori status will be excluded from the PP analysis.

Safety analysis population: The patients with missing safety data in ITT population will excluded from the safety analysis.

10.3 Statistical methods

Comparative superiority of the two groups was assessed through hypothesis testing (one-sided Z test) and derivation of a two-sided 95% confidence interval (CI) of difference based on H. pylori eradication rate. If p-value of the testing less than 0.025 and the lower bound of the 95% CI greater than zero, superiority of susceptibility-based therapy over empirical therapy could be concluded.

Between-group differences were evaluated using Student's t-test for continuous variables and Pearson's χ2/corrected χ2 or Fisher's exact test for categorical variables, as appropriate. All p-values were two-sided except the testing of superiority, and were considered statistically significant if p-value less than 0.05.

Subgroup analyses of eradication efficacy were performed based on the results of antibiotic susceptibility test and compliance assessment.

11 Ethical principles

The study is registered in ClinicalTrials.gov. The study protocol is approved by the Ethics Committee at all institutions, and the study will be performed according to good clinical practice and the Declaration of Helsinki.

Before each patient is selected into the study, the physician of the study have the responsibility to tell patients or their designated agents the purpose, process, completion and comprehensively introduce possible adverse reactions, risks to bear, possible benefits and other information all-round and detailed, making the patients know their rights. We are ordered to inform the patients of having the right to decide whether to participate in the study, as well as the right to withdraw the study at any time without any discrimination. The patients or their legal representatives should sign the informed consent after carefully reading and fully understanding, and retain the signature page of the copy.

12 References

1. Malfertheiner P, Megraud F, O'Morain CA, et al. Management of Helicobacter pylori infection—the Maastricht IV/Florence Consensus Report. Gut 2012;61:646–64.

2. Thung I, Aramin H, Vavinskaya V, Gupta S, Park JY, Crowe SE, et al. Review article: the global emergence of Helicobacter pylori antibiotic resistance. Aliment Pharmacol Ther. 2016 Feb 1;43(4):514–33.

3. Su P, Li Y, Li H, Zhang J, Lin L, Wang Q, et al. Antibiotic Resistance of Helicobacter Pylori Isolated in the Southeast Coastal Region of China. Helicobacter. 2013 Aug 1;18(4):274–9.

4. Bai P, Zhou LY, Xiao XM, Luo Y, Ding Y. Susceptibility of Helicobacter pylori to antibiotics in Chinese patients. J Dig Dis. 2015 Aug;16(8):464–70.

5. Zhang Y-X, Zhou L-Y, Song Z-Q, Zhang J-Z, He L-H, Ding Y. Primary antibiotic resistance of Helicobacter pylori strains isolated from patients with dyspeptic symptoms in Beijing: A prospective serial study. World J Gastroenterol WJG. 2015 Mar 7;21(9):2786–92.

6. Wang B, Lv Z-F, Wang Y-H, Wang H, Liu X-Q, Xie Y, et al. Standard triple therapy for Helicobacter pylori infection in China: A meta-analysis. World J Gastroenterol. 2014 Oct 28;20(40):14973–85.

7. Wenzhen Y, Yumin L, Quanlin G, Kehu Y, Lei J, Donghai W, et al. Is Antimicrobial Susceptibility Testing Necessary Before First-line Treatment for Helicobacter pylori Infection? -Meta-analysis of Randomized Controlled Trials-. Intern Med. 2010;49(12):1103–9.

8. López-Góngora S, Puig I, Calvet X, Villoria A, Baylina M, Muñoz N, et al. Systematic review and meta-analysis: susceptibility-guided versus empirical antibiotic treatment for Helicobacter pylori infection. J Antimicrob Chemother. 2015 Sep;70(9):2447–55.

9. Chen H, Dang Y, Zhou X, Liu B, Liu S, Zhang G. Tailored Therapy Versus Empiric Chosen Treatment for Helicobacter pylori Eradication: A Meta-Analysis. Medicine (Baltimore). 2016 Feb;95(7):e2750.

10. Zhang W, Chen Q, Liang X, Liu W, Xiao S, Graham DY, et al. Bismuth, lansoprazole, amoxicillin and metronidazole or clarithromycin as first-line Helicobacter pylori therapy. Gut. 2015 Nov;64(11):1715–20.

11. Zhou L, Zhang J, Song Z, He L, Li Y, Qian J, et al. Tailored versus Triple plus Bismuth or Concomitant Therapy as Initial Helicobacter pylori Treatment: A Randomized Trial. Helicobacter. 2016 Apr 1;21(2):91–9.

12. Graham DY, Dore MP. Helicobacter pylori therapy: a paradigm shift. Expert Rev Anti Infect Ther. 2016 Jun;14(6):577–85.

Signature Page

Protocol Title:

Efficacy of Antibiotic Susceptibility-based Tailored Versus Empiric Therapy for Helicobacter pylori First-line Treatment：a Multicenter Randomized Controlled Trial

After reviewing the protocol, each investigator and statistician signed this page as an attachment.

Investigator:

I will earnestly fulfill my duties as an investigator in accordance with the *Chinese Good Clinical Practice* (GCP).

This study will be conducted in accordance with the principles of morality, ethics and science laid down in the Helsinki Declaration and the Chinese GCP. I agree to carry out this clinical trial in accordance with this protocol.

I will be responsible for making timely and appropriate medical decisions for adverse events that occur in subjects during the study period. I know the requirements for correctly reporting serious adverse events and I will record and report these events.

I guarantee that the data will be accurately, completely, timely, and legally filled in the Case Report Form. I will accept the inspections to ensure the quality of the study.

I agree that the findings of the study are published.

Principal Investigator: Hong Lu

Institution: Renji Hospital, School of Medicine, Shanghai Jiao Tong University,

Shanghai, China.

Signature: _____

Co-investigator: Yunwei Sun

Institution: Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

Signature: _____

Co-investigator: Hong Gao

Institution: Zhongshan Hospital, Fudan University, Shanghai, China

Signature: _____

Co-investigator: Yan Zhan

Institution: Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai, China.

Signature: _____

Co-investigator: Gang Xu

Institution: Department of Gastroenterology and Hepatology, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

Signature: _____

Statistical unit

We will conduct statistical analysis based on the Chinese "guideline of Biostatistics for Clinical Trials of Chemical Drugs and Biological Products" and related regulations.

Statistician: Yanyan Song

Institution: Institute of Medical Sciences, Shanghai Jiao Tong University School of Medicine, Shanghai, China

Signature: _____

Summary of protocol changes

Change	Original	Final
Title	Efficacy of Clarithromycin Susceptibility-based Tailored Versus Empiric Therapy for Helicobacter pylori First-line Treatment： a Multicenter Randomized Controlled Trial	Efficacy of **Antibiotic** Susceptibility-based Tailored Versus Empiric Therapy for Helicobacter pylori First-line Treatment： a Multicenter Randomized Controlled Trial
2.2 The secondary objective	(1) To compare the safety and compliance of tailored therapy with empiric modified bismuth quadruple therapy; (2) To evaluate the impact of clarithromycin resistance on eradication rates; (3) To evaluate the diagnosis accuracy of molecular genetic assays in clarithromycin resistance identification (4) To evaluate the cost-effectiveness of tailored therapy in treating	(1) To compare the safety and compliance of tailored therapy with empiric modified bismuth quadruple therapy; (2) To evaluate the impact of antibiotic resistance on eradication rates; (3) To evaluate the cost-effectiveness of susceptibility-based tailored therapy in treating naïve patients with H. pylori infection.

	naïve patients with H. pylori infection.	
6.2 Intervention	**(1) Empirical therapy** After review of clarithromycin-taking medical history, if have used clarithromycin, give esomeprazole 20mg bid, bismuth potassium citrate 600mg bid, amoxicillin 1000mg bid and metronidazole 400mg bid for 14 days; if have not used clarithromycin, give esomeprazole 20mg bid, bismuth potassium citrate 600mg bid, amoxicillin 1000mg bid and clarithromycin 500mg bid for 14 days. **(2) Tailored therapy** After molecular genetic assays for identification of resistance to clarithromycin of Helicobacter pylori from biopsy samples, if resistance to clarithromycin, give esomeprazole 20mg bid, bismuth potassium citrate 600mg bid, amoxicillin 1000mg bid and metronidazole 400mg bid for 14 days; if susceptible to clarithromycin, give esomeprazole 20mg bid, bismuth potassium citrate 600mg bid, amoxicillin 1000mg bid and clarithromycin 500mg bid for 14 days.	**(1) Empirical therapy** Esomeprazole 20 mg, bismuth potassium citrate 600 mg taken twice a day, amoxicillin 1 g, and metronidazole 500 mg taken three times a day for 14 days. **(2) Tailored therapy** Tailored therapy is based on antimicrobial susceptibility of clarithromycin, metronidazole and levofloxacin. a) If the H. pylori strain of one patient was sensitive to clarithromycin, clarithromycin triple therapy (EAC) will be given: esomeprazole 20 mg, amoxicillin 1 g, and clarithromycin 500 mg taken twice a day; b) If the strain was resistant to clarithromycin but sensitive to metronidazole, metronidazole triple therapy (EAM) will be given: esomeprazole 20 mg, amoxicillin 1 g, and metronidazole 500 mg taken twice a day. c) If the strain was resistant to both clarithromycin and metronidazole but sensitive to levofloxacin, levofloxacin triple therapy (EAL) will be given:

		esomeprazole 20 mg, amoxicillin 1 g taken twice a day, and levofloxacin 500 mg taken once a day. d) If the strain was resistant to all three antibiotics, bismuth quadruple therapy (EBA_2M_4) will be given: esomeprazole 20 mg, bismuth potassium citrate 600 mg, amoxicillin 1 g taken twice a day, and metronidazole 500 mg taken four times a day. Study treatment regimens will be all given 14 days.
6.3 Drugs	**Esomeprazole**, 20mg/tablet, AstraZeneca AB, Sodertalje, Sweden. **Bismuth potassium citrate**, 300mg/capsule (110mg elemental bismuth), Dawnrays Pharmaceutical Limited, Suzhou, Jiangsu, China. **Amoxicillin**, 250mg/capsule, Ruiyang Pharmaceutical Co. Ltd., Shandong, China. **Clarithromycin**, 250mg/tablet, Shanghai Abbott Laboratories Co. Ltd., Shanghai, China. **Metronidazole**, 200mg/tablet, Shanghai Xinyi Wanxiang	**Esomeprazole**, 20mg/tablet, AstraZeneca AB, Sodertalje, Sweden. **Bismuth potassium citrate**, 300mg/capsule (110mg elemental bismuth), Dawnrays Pharmaceutical Limited, Suzhou, Jiangsu, China. **Amoxicillin**, 250mg/capsule, Ruiyang Pharmaceutical Co. Ltd., Shandong, China. **Clarithromycin**, 250mg/tablet, Shanghai Abbott Laboratories Co. Ltd., Shanghai, China. **Metronidazole**, 200mg/tablet, Shanghai Xinyi Wanxiang Pharmaceutical

	Pharmaceutical Industry Company Limited, Shanghai, China.	Industry Company Limited, Shanghai, China. **Levofloxacin, 500mg/tablet, Daiichi Sankyo Pharmaceutical Beijing Co., Ltd., Beijing, China.**
7. Antibiotic Susceptibility Testing	Tissue samples will be obtained from gastroscopic biopsy. DNAs will be isolated from biopsy specimens and mutation of clarithromycin resistance will be identified by standard molecular (real-time PCR) instrument systems and The *Helicobacter pylori* Analyte Specific Reagents (BIOLINE USA Inc., MA, USA) according to the manufacturer's guidelines. The biopsy specimens will be cultured and maintained on brain heart infusion agar medium (OXOID, Basingstoke, UK) containing 5% defibrinated sheep blood under microaerophilic conditions (85% N2, 10% CO2, 5% O2) at 37℃.	Tissue samples will be obtained from gastroscopic biopsy. The biopsy specimens will be cultured and maintained on brain heart infusion agar medium (OXOID, Basingstoke, UK) containing 5% defibrinated sheep blood under microaerophilic conditions (85% N2, 10% CO2, 5% O2) at 37℃.
9.3 Other Pre-specified Outcome	(1) H. pylori eradication rate of patients with clarithromycin resistance strains (2) H. pylori eradication rate of patients with clarithromycin sensitive	(1) Medical cost per patient of tailored or empiric therapy Medical cost of one patient is overall cost including visit, endoscopy, H. pylori culture, susceptibility testing, eradication treatment, and adverse event

Measures:	strains (3) Sensitivity, specificity, positive predictive value and negative predictive value of molecular genetic assays in clarithromycin resistance identification (4) Medical cost per patient of tailored or empiric therapy (5) Ratio of medical cost to H. pylori eradication rate of each therapy (6) Ratio of incremental medical cost to incremental H. pylori eradication rate of tailored compared with empiric therapy	treatment. (2) Ratio of medical cost to H. pylori eradication rate of each therapy (3)Ratio of incremental medical cost to incremental H. pylori eradication rate of tailored compared with empiric therapy
10.1 Sample size	Assuming 95.7% eradication rate of tailored therapy in our trial, 88.8% eradication rate of empirical therapy, a superiority margin of >0, a power of 80%, an alpha of 0.05 (one-sided), a ratio of 3:1, at least 340 subjects in susceptibility-based therapy and 114 subjects in empirical therapy will be required to show superiority of susceptibility-based therapy over empirical therapy on the basis of H. pylori eradication rate. Taking into	Assuming 96.5% eradication rate of tailored therapy in our trial, 88.9% eradication rate of empirical therapy, a superiority margin of >0, a power of 80%, an alpha of 0.05 (one-sided), a ratio of 3:1, at least 260 subjects in susceptibility-based therapy and 87 subjects in empirical therapy will be required to show superiority of susceptibility-based therapy over empirical therapy on the basis of H. pylori eradication rate. Taking into consideration

	consideration of 10% lost to follow-up, at least 500 participants (374 for tailored therapy and 126 for empiric therapy) is expected to be recruited for the study.	of 10% lost to follow-up, at least 382 participants (286 for tailored therapy and 96 for empiric therapy) is expected to be recruited for the study

Original Statistical Analysis Plan

This statistical analysis plan includes the details of study sample size calculation and statistical methods for analysis of outcomes and safety.

Table of Contents

1. Sample size estimation

Assuming 95.7% eradication rate of tailored therapy in our trial, 88.8% eradication rate of empirical therapy, a superiority margin of >0, a power of 80%, an alpha of 0.05 (one-sided), a ratio of 3:1, at least 340 subjects in susceptibility-based therapy and 114 subjects in empirical therapy will be required to show superiority of susceptibility-based therapy over empirical therapy on the basis of H. pylori eradication rate. Taking into consideration of 10% lost to follow-up, at least 500 participants (374 for tailored therapy and 126 for empiric therapy) is expected to be recruited for the study.

2. Definition of populations for analysis

2.1 Intention--to-treat (ITT) Analysis population

According to the Intention-to-treat principle: All randomized patients will be included in the ITT analysis. Patients who did not return for a follow-up 13C-UBT will be recorded as treatment failures.

2.2 Per-protocol (PP) analysis population

All individuals who violated the study protocol, such as patients not taking at least 80% of treatment drugs, or with unknown post-treatment H. pylori status will be excluded from the PP analysis.

2.3 Safety analysis population

The patients with missing safety data in ITT population will excluded from the safety analysis.

3. Evaluation of baseline characteristics

3.1 presentation of baseline characteristics

Variables of baseline characteristics collected are age (years), gender (male/female), diagnosis (Dyspepsia/PUD), clarithromycin-taking history, 23S rRNA mutation, antibiotic resistance pattern, poor adherence, and loss of follow-up. The following table shows the types and presentation of variables.

Variable	Type of variable	Presentation
Age (years)	Continuous	Mean ± standard deviation (range)
Gender (male/female)	Categorical	Number (percentage)
Diagnosis (Dyspepsia/PUD)	Categorical	Number (percentage)
Clarithromycin-taking history	Categorical	Number (percentage)
23S rRNA mutation	Categorical	Number (percentage)
Antibiotic resistance pattern（Phenotypic）		
Clarithromycin, n (%)	Categorical	Number (percentage)
Metronidazole, n (%)	Categorical	Number (percentage)
Levofloxacin, n (%)	Categorical	Number (percentage)
All-susceptible, n (%)	Categorical	Number (percentage)
Triple-resistant, n (%)	Categorical	Number (percentage)
Poor adherence	Categorical	Number
Loss of follow-up	Categorical	Number

3.2 Statistical methods for baseline characteristics analyses

Comparisons of baseline characteristics between the tailored therapy and empiric therapy will be performed in the ITT analysis populations. Comparisons between the both groups will be performed using the Pearson's $\chi 2$, corrected $\chi 2$ or Fisher's exact test for categorical variables and using the Student t test for continuous variables.

4. Evaluation of primary outcomes

4.1 *Helicobacter pylori* eradication rate

Six weeks after completion of therapy, H. pylori eradication success is defined as negative result from urea breath test (<4‰). If patients would be lost to follow-up urea breath testing, they will be scored as treatment failures in the ITT analysis.

The eradication rate is defined as the number of patients who successful eradicate *H. pylori* divided by the total number of population for analysis. These data are presented with number, percentage, and 95% confidence interval (CI).

4.2 Testing the differences of eradication rates

Testing the differences of eradication rates between the tailored therapy and empiric therapy will be performed using the Pearson's $\chi 2$, corrected $\chi 2$ or Fisher's exact test in the ITT and PP analysis populations.

4.3 Testing the superiority of the tailored therapy

Testing the superiority of tailored therapy group to empiric therapy group will be performed in the ITT and PP analysis populations using hypothesis testing (one-sided Z test) and derivation of a two-sided 95% confidence interval (CI) of difference based on H. pylori eradication rate. If p-value of the testing less than 0.025 and the lower bound of the 95% CI greater than zero, superiority of susceptibility-based therapy over empirical therapy could be concluded.

5. Evaluation of secondary outcomes

5.1 Rate of adverse events

During the 14-day treatment period, the subjects will keep a diary to score any possible side effects or discomforts. The subjects will be asked to grade the severity of adverse events according to their influence on daily activities, experienced as “mild” (transient and well tolerated), “moderate” (causing discomfort and partially interfering with daily activities), or “severe” (causing considerable interference with daily activities). The side effect score recorded is based on the most severe event. Rate of adverse events is defined as the number of patients with any adverse event divided by the total number of population for analysis. These data are presented with number, percentage.

Comparisons of presence of each adverse event and any adverse event between the tailored therapy and empiric therapy will be performed in the safety analysis populations using the Pearson’s $\chi 2$, corrected $\chi 2$ or Fisher’s exact test.

5.2 Compliance rate

Compliance was defined as either poor when they had taken less than 80% of the total medication or good when they had taken more than 80% medication. Compliance rate is defined as the number of patients with good compliance divided by the total number of population for analysis. These data are presented with number, percentage.

Comparisons of presence of each adverse event and any adverse event between the tailored therapy and empiric therapy will be performed in the ITT analysis populations using the Pearson’s $\chi 2$, corrected $\chi 2$ or Fisher’s exact test.

5.3 Other Pre-specified Outcome Measures:

(1) Medical cost per patient of tailored or empiric therapy

Medical cost of one patient is overall cost including visit, endoscopy, H. pylori culture, susceptibility testing, eradication treatment, and adverse event treatment.

Comparisons of medical cost between the tailored therapy and empiric therapy will be performed in the PP analysis populations using Student t test.

(2) Ratio of medical cost to H. pylori eradication rate of each therapy

Ratio of medical cost to H. pylori eradication rate of each therapy is defined as average medical cost of the therapy divided by H. pylori eradication rate of one therapy.

(3)Ratio of incremental medical cost to incremental H. pylori eradication rate of tailored compared with empiric therapy

If one therapy cost less and achieved higher eradication rate than the other one, it will be referred to as dominant strategy. If one therapy yielded higher eradication rate but also cost more, an incremental cost-effectiveness ratio (ICER) will be calculated. Obviously, the tailored therapy will cost more because of additional bacterial culture and antibiotic susceptibility testing. So, if eradication rate of the tailored therapy was higher, the ICER of the tailored therapy will be calculated as the ratio of incremental medical cost to incremental H. pylori eradication rate of tailored compared with empiric therapy (in terms of RMB per incremental eradication percentage). if eradication rate of the tailored therapy was equal to or lower than the empiric therapy, the tailored therapy will not be more cost-effective option and the economic evaluation will not be conducted.

6. Additional analyses

6.1 Antibiotic resistance rate

Minimal inhibitory concentrations (MIC) of clarithromycin >2 μg/mL, metronidazole >8 μg/mL, levofloxacin >2 μg/mL is defined as resistance breakpoints. Antibiotic resistance rate is defined as the number of patients with antibiotic resistant strains divided by the total number of population for analysis. These data are presented

with number, percentage.

6.2 Effect of antibiotic resistance on eradication rate

Comparisons of eradication rate of one therapy between antibiotic susceptible and resistant strains will be performed in the PP analysis populations using the Pearson's $\chi 2$, corrected $\chi 2$ or Fisher's exact test.

Additional comparisons of eradication rate of antibiotic resistant strains between the tailored therapy and the empiric therapy will be performed in the PP analysis populations using the Pearson's $\chi 2$, corrected $\chi 2$ or Fisher's exact test.

6.3 Effect of 23S rRNA mutation on eradication rate

Comparisons of eradication rate of clarithromycin-containing therapy between strains with 23S rRNA mutation and those without 23S rRNA mutation will be performed in the PP analysis populations using the Pearson's $\chi 2$, corrected $\chi 2$ or Fisher's exact test.

6.4 Sensitivity, specificity, positive predictive value and negative predictive value of molecular genetic assays in clarithromycin resistance identification

Using culture based antibiotic susceptibility testing as the gold standard, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 23S rRNA mutation in identification of *H. pylori* resistance to clarithromycin will be estimated.

$$Sensitivty = \frac{\text{number of true positives}}{\text{number of true positives} + \text{number of false negatives}}$$

$$Specificity = \frac{\text{number of true negatives}}{\text{number of true negatives} + \text{number of false positives}}$$

$$PPV = \frac{\text{number of true positives}}{\text{number of true positives} + \text{number of false positives}}$$

$$NPV = \frac{\text{number of true negatives}}{\text{number of true negatives} + \text{number of false negatives}}$$

True positives: the strains with 23S rRNA mutation and MIC of clarithromycin >2

μg/mL

False positives: the strains with 23S rRNA mutation but MIC of clarithromycin ≤2 μg/mL

True negatives: the strains without 23S rRNA mutation and MIC of clarithromycin ≤2 μg/mL

False negatives: the strains without 23S rRNA mutation but MIC of clarithromycin >2 μg/mL

6.5 Effect of presence of adverse events on compliance.

Comparisons of compliance rate of one therapy between presence and absence of adverse event will be performed in the safety analysis populations using the Pearson's $\chi 2$, corrected $\chi 2$ or Fisher's exact test.

6.6 Effect of compliance on eradication rate

Comparisons of eradication rate of one therapy between good and poor compliance will be performed in the ITT analysis populations who had UBT results using the Pearson's $\chi 2$, corrected $\chi 2$ or Fisher's exact test.

7. Table Design

Table 1. Baseline characteristics

Variables	Tailored therapy	Empiric therapy	p value
Age, years (range)			
Gender (male/female)			
Diagnosis (Dyspepsia/PUD)			
Clarithromycin-taking history			
23S rRNA mutation			
Antibiotic resistance pattern			
Clarithromycin, n (%)			
Metronidazole, n (%)			

Levofloxacin, n (%)	
All-susceptible, n (%)	
Triple-resistant, n (%)	
Poor adherence*	
Loss of follow-up	

NUD, non-ulcer dyspepsia; PUD, healed peptic ulcer disease.

* Poor adherence, took less than 80% of drugs

Table 2. Eradication rate of each group in ITT and PP analysis

Analysis	**Tailored therapy**	**Empiric therapy**	**Difference**	***p* value for inequality***	***p* value for superiority ****
ITT (%, n/N)					
95% CI					
PP (%, n/N)					
95% CI					

CI= confidence interval; ITT = intention-to-treat; PP = per-protocol.

* The p values were for testing the difference between tailored therapy group and empiric therapy group.

** The p values were for testing the superiority of tailored therapy group to empiric therapy group.

Table 3. antibiotic resistance pattern of ITT population

antibiotic resistance pattern (CLA-MET-LEV)	**ITT population**	**Tailored therapy**	**Empiric therapy**
All susceptible			
S-S-S			
Single resistant			
R-S-S			
S-R-S			
S-S-R			
Dual resistant			
R-R-S			

R-S-R	
S-R-R	
Triple resistant	
R-R-R	

CLA, clarithromycin; LEV, levofloxacin; MET, metronidazole; R, resistant; S, susceptible.

Table 4. Eradication rates of tailored and empiric therapy in the presence of antibiotic resistance in the per-protocol population

PP population	Tailored therapy	Empiric therapy	*p* value
23S rRNA mutation (genotypic)			
Susceptible			
Resistant			
Clarithromycin resistance (phenotypic)			
Susceptible			
Resistant			
Metronidazole resistance (phenotypic)			
Susceptible			
Resistant			

Table 5. Adverse events and adherence in the treatment groups

Variables	Tailored therapy	Empiric therapy	*p* value*
Total, n/N (%)			
AE grade			
None/Mild/Moderate/Severe			
AE variety			
Taste alteration			
Dyspepsia			
……			
……			
……			

Skin rash
Discontinued drugs because of AEs
Good Adherence (Took at least 80% of drugs)
Subjects without AEs
Subjects with AEs
Total

AE, adverse event.

Final Statistical Analysis Plan

This statistical analysis plan includes the details of study sample size calculation and statistical methods for analysis of outcomes and safety.

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1. Sample size estimation

Assuming 96.5% eradication rate of susceptibility-based therapy in our trial, 88.9% eradication rate of empirical therapy, a superiority margin of >0, a power of 80%, an alpha of 0.05 (one-sided), a ratio of 3:1, at least 260 subjects in susceptibility-based therapy and 87 subjects in empirical therapy will be required to show superiority of susceptibility-based therapy over empirical therapy on the basis of H. pylori eradication rate. Taking into consideration of 10% lost to follow-up, at least 382 participants (286 for tailored therapy and 96 for empiric therapy) is expected to be recruited for the study.

2. Definition of populations for analysis

2.1 Intention--to-treat (ITT) Analysis population

According to the Intention-to-treat principle: All randomized patients will be included in the ITT analysis. Patients who did not return for a follow-up 13C-UBT will be recorded as treatment failures.

2.2 Per-protocol (PP) analysis population

All individuals who violated the study protocol, such as patients not taking at least 80% of treatment drugs, or with unknown post-treatment H. pylori status will be excluded from the PP analysis.

2.3 Safety analysis population

The patients with missing safety data in ITT population will excluded from the safety analysis.

3. Evaluation of baseline characteristics

3.1 presentation of baseline characteristics

Variables of baseline characteristics collected are age (years), gender (male/female), diagnosis (Dyspepsia/PUD), antibiotic resistance pattern, poor adherence, and loss of follow-up. The following table shows the types and presentation of variables.

Variable	Type of variable	Presentation
Age (years)	Continuous	Mean ± standard deviation (range)
Gender (male/female)	Categorical	Number (percentage)
Diagnosis (Dyspepsia/PUD)	Categorical	Number (percentage)
Antibiotic resistance pattern		
Clarithromycin, n (%)	Categorical	Number (percentage)
Metronidazole, n (%)	Categorical	Number (percentage)
Levofloxacin, n (%)	Categorical	Number (percentage)
All-susceptible, n (%)	Categorical	Number (percentage)
Triple-resistant, n (%)	Categorical	Number (percentage)
Poor adherence*	Categorical	Number
Loss of follow-up	Categorical	Number

3.2 Statistical methods for baseline characteristics analyses

Comparisons of baseline characteristics between the tailored therapy and empiric therapy will be performed in the ITT analysis populations. Comparisons between the both groups will be performed using the Pearson's $\chi 2$, corrected $\chi 2$ or Fisher's exact test for categorical variables and using the Student t test for continuous variables.

4. Evaluation of primary outcomes

4.1 *Helicobacter pylori* eradication rate

Six weeks after completion of therapy, H. pylori eradication success is defined as negative result from urea breath test (<4‰). If patients would be lost to follow-up urea breath testing, they will be scored as treatment failures in the ITT analysis.

The eradication rate is defined as the number of patients who successful eradicate *H. pylori* divided by the total number of population for analysis. These data are presented with number, percentage, and 95% confidence interval (CI).

4.2 Testing the differences of eradication rates

Testing the differences of eradication rates between the tailored therapy and empiric therapy will be performed using the Pearson's $\chi 2$, corrected $\chi 2$ or Fisher's exact test in the ITT and PP analysis populations.

4.3 Testing the superiority of the tailored therapy

Testing the superiority of tailored therapy group to empiric therapy group will be performed in the ITT and PP analysis populations using hypothesis testing (one-sided Z test) and derivation of a two-sided 95% confidence interval (CI) of difference based on H. pylori eradication rate. If p-value of the testing less than 0.025 and the lower bound of the 95% CI greater than zero, superiority of susceptibility-based therapy over empirical therapy could be concluded.

5. Evaluation of secondary outcomes

5.1 Rate of adverse events

During the 14-day treatment period, the subjects will keep a diary to score any possible side effects or discomforts. The subjects will be asked to grade the severity of adverse events according to their influence on daily activities, experienced as "mild" (transient

and well tolerated), “moderate” (causing discomfort and partially interfering with daily activities), or “severe” (causing considerable interference with daily activities). The side effect score recorded is based on the most severe event. Rate of adverse events is defined as the number of patients with any adverse event divided by the total number of population for analysis. These data are presented with number, percentage.

Comparisons of presence of each adverse event and any adverse event between the tailored therapy and empiric therapy will be performed in the safety analysis populations using the Pearson’s $\chi 2$, corrected $\chi 2$ or Fisher’s exact test.

5.2 Compliance rate

Compliance was defined as either poor when they had taken less than 80% of the total medication or good when they had taken more than 80% medication. Compliance rate is defined as the number of patients with good compliance divided by the total number of population for analysis. These data are presented with number, percentage.

Comparisons of presence of each adverse event and any adverse event between the tailored therapy and empiric therapy will be performed in the ITT analysis populations using the Pearson’s $\chi 2$, corrected $\chi 2$ or Fisher’s exact test.

5.3 Other Pre-specified Outcome Measures:

(1) Medical cost per patient of tailored or empiric therapy

Medical cost of one patient is overall cost including visit, endoscopy, H. pylori culture, susceptibility testing, eradication treatment, and adverse event treatment.

Comparisons of medical cost between the tailored therapy and empiric therapy will be performed in the PP analysis populations using Student t test.

(2) Ratio of medical cost to H. pylori eradication rate of each therapy

Ratio of medical cost to H. pylori eradication rate of each therapy is defined as average medical cost of the therapy divided by H. pylori eradication rate of one therapy.

(3)Ratio of incremental medical cost to incremental H. pylori eradication rate of tailored compared with empiric therapy

If one therapy cost less and achieved higher eradication rate than the other one, it will be referred to as dominant strategy. If one therapy yielded higher eradication rate but also cost more, an incremental cost-effectiveness ratio (ICER) will be calculated. Obviously, the tailored therapy will cost more because of additional bacterial culture and antibiotic susceptibility testing. So, if eradication rate of the tailored therapy was higher, the ICER of the tailored therapy will be calculated as the ratio of incremental medical cost to incremental H. pylori eradication rate of tailored compared with empiric therapy (in terms of RMB per incremental eradication percentage). if eradication rate of the tailored therapy was equal to or lower than the empiric therapy, the tailored therapy will not be more cost-effective option and the economic evaluation will not be conducted.

6. Additional analyses

6.1 Antibiotic resistance rate

Minimal inhibitory concentrations (MIC) of clarithromycin >2 μg/mL, metronidazole >8 μg/mL, levofloxacin >2 μg/mL is defined as resistance breakpoints. Antibiotic resistance rate is defined as the number of patients with antibiotic resistant strains divided by the total number of population for analysis. These data are presented with number, percentage.

6.2 Effect of antibiotic resistance on eradication rate

Comparisons of eradication rate of one therapy between antibiotic susceptible and

resistant strains will be performed in the PP analysis populations using the Pearson's $\chi 2$, corrected $\chi 2$ or Fisher's exact test.

Additional comparisons of eradication rate of antibiotic resistant strains between the tailored therapy and the empiric therapy will be performed in the PP analysis populations using the Pearson's $\chi 2$, corrected $\chi 2$ or Fisher's exact test.

6.3 Effect of presence of adverse events on compliance.

Comparisons of compliance rate of one therapy between presence and absence of adverse event will be performed in the safety analysis populations using the Pearson's $\chi 2$, corrected $\chi 2$ or Fisher's exact test.

6.4 Effect of compliance on eradication rate

Comparisons of eradication rate of one therapy between good and poor compliance will be performed in the ITT analysis populations who had UBT results using the Pearson's $\chi 2$, corrected $\chi 2$ or Fisher's exact test.

7. Table Design

Table 1. Baseline characteristics

Variables	Tailored therapy	Empiric therapy	p value
Age, years (range)			
Gender (male/female)			
Diagnosis (Dyspepsia/PUD)			
Antibiotic resistance pattern			
Clarithromycin, n (%)			
Metronidazole, n (%)			
Levofloxacin, n (%)			
All-susceptible, n (%)			
Triple-resistant, n (%)			
Poor adherence*			
Loss of follow-up			

NUD, non-ulcer dyspepsia; PUD, healed peptic ulcer disease.

* Poor adherence, took less than 80% of drugs

Table 2. Eradication rate of each group in ITT and PP analysis

Analysis	Tailored therapy	Empiric therapy	Difference	*p* value for inequality*	*p* value for superiority **
ITT (%, n/N)					
95% CI					
PP (%, n/N)					
95% CI					

CI= confidence interval; ITT = intention-to-treat; PP = per-protocol.

* The p values were for testing the difference between tailored therapy group and empiric therapy group.

** The p values were for testing the superiority of tailored therapy group to empiric therapy group.

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antibiotic resistance pattern (CLA-MET-LEV)	ITT population	Tailored therapy	Empiric therapy
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Single resistant			
R-S-S			
S-R-S			
S-S-R			
Dual resistant			
R-R-S			
R-S-R			
S-R-R			
Triple resistant			
R-R-R			

CLA, clarithromycin; LEV, levofloxacin; MET, metronidazole; R, resistant; S, susceptible.

Table 4. Eradication rates of tailored and empiric therapy in the presence of antibiotic resistance in the per-protocol population

PP population	Tailored therapy	Empiric therapy	*p* value
Clarithromycin resistance			
Susceptible			
Resistant			
Metronidazole resistance			
Susceptible			
Resistant			
Levofloxacin resistance			
Susceptible			
Resistant			
Resistance pattern (Clarithromycin-Metronidazole-Levofloxacin)			
S-X-X			
R-S-X			
R-R-S			
R-R-R			

R=resistant; S=susceptible; X=resistant or susceptible.

Table 5. Adverse events and adherence in the treatment groups

Variables	Tailored therapy	Empiric therapy	*p* value*
Total, n/N (%)			
AE grade			
None/Mild/Moderate/Severe			
AE variety			
Taste alteration			
Dyspepsia			
……			
……			
……			

Skin rash
Discontinued drugs because of AEs
Good Adherence (Took at least 80% of drugs)
Subjects without AEs
Subjects with AEs
Total

AE, adverse event.

Summary of Statistical Analysis Plan Changes

Change	Original	Final
1.Sample size estimation	Assuming 95.7% eradication rate of tailored therapy in our trial, 88.8% eradication rate of empirical therapy, a superiority margin of >0, a power of 80%, an alpha of 0.05 (one-sided), a ratio of 3:1, at least 340 subjects in susceptibility-based therapy and 114 subjects in empirical therapy will be required to show superiority of susceptibility-based therapy over empirical therapy on the basis of H. pylori eradication rate. Taking into consideration of 10% lost to follow-up, at least 500 participants (374 for tailored therapy and 126 for empiric therapy) is expected to be recruited for the study.	Assuming 96.5% eradication rate of tailored therapy in our trial, 88.9% eradication rate of empirical therapy, a superiority margin of >0, a power of 80%, an alpha of 0.05 (one-sided), a ratio of 3:1, at least 260 subjects in susceptibility-based therapy and 87 subjects in empirical therapy will be required to show superiority of susceptibility-based therapy over empirical therapy on the basis of H. pylori eradication rate. Taking into consideration of 10% lost to follow-up, at least 382 participants (286 for tailored therapy and 96 for empiric therapy) is expected to be recruited for the study
3.1 presentation of	Variables of baseline characteristics collected are age (years), gender (male/female), diagnosis (Dyspepsia/PUD), clarithromycin-taking	Variables of baseline characteristics collected are age (years), gender (male/female), diagnosis (Dyspepsia/PUD), ~~**clarithromycin-taking**~~

baseline characteristics	history, 23S rRNA mutation, antibiotic resistance pattern, poor adherence, and loss of follow-up. The following table shows the types and presentation of variables.	~~**history, 23S rRNA mutation,**~~ antibiotic resistance pattern, poor adherence, and loss of follow-up. The following table shows the types and presentation of variables.
6. Additional analyses	**6.3 Effect of 23S rRNA mutation on eradication rate** Comparisons of eradication rate of clarithromycin-containing therapy between strains with 23S rRNA mutation and those without 23S rRNA mutation will be performed in the PP analysis populations using the Pearson's $\chi 2$, corrected $\chi 2$ or Fisher's exact test. **6.4 Sensitivity, specificity, positive predictive value and negative predictive value of molecular genetic assays in clarithromycin resistance identification** Using culture based antibiotic susceptibility testing as the gold standard, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 23S rRNA mutation in identification of *H. pylori* resistance to clarithromycin will be	~~**6.3 Effect of 23S rRNA mutation on eradication rate**~~ **(Deleted)** ~~Comparisons of eradication rate of clarithromycin-containing therapy between strains with 23S rRNA mutation and those without 23S rRNA mutation will be performed in the PP analysis populations using the Pearson's $\chi 2$, corrected $\chi 2$ or Fisher's exact test.~~ ~~**6.4 Sensitivity, specificity, positive predictive value and negative predictive value of molecular genetic assays in clarithromycin resistance identification**~~ **(Deleted)** ~~Using culture based antibiotic susceptibility testing as the gold standard, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 23S rRNA mutation in identification of *H. pylori*~~

	estimated.	~~resistance to clarithromycin will be estimated.~~
7. Table Design	**Table 1.** Baseline characteristics **Table 4.** Eradication rates of tailored and empiric therapy in the presence of antibiotic resistance in the per-protocol population	In Table 1, clarithromycin-taking history and 23S rRNA mutation were deleted. In Table 4, subgrouping according to 23S rRNA mutation was deleted, and subgrouping according to levofloxacin resistance and antibiotic resistance pattern (Clarithromycin-Metronidazole-Levofloxacin) was added.