Molecular testing-guided therapy versus susceptibility testing-guided therapy in first-line and third-line *Helicobacter pylori* eradication: two multicentre, open-label, randomised controlled, non-inferiority trials

Mei-Jyh Chen, Po-Yueh Chen*, Yu-Jen Fang, Ming-Jong Bair, Chieh-Chang Chen, Chien-Chuan Chen, Tsung-Hua Yang, Ji-Yuh Lee, Chien-Chun Yu, Chia-Chi Kuo, Min-Chin Chiu, Chu-Kuang Chou, Chi-Yi Chen, Wen-Hao Hu, Min-Horn Tsai, Yao-Chun Hsu, Chia-Tung Shun, Jiing-Chyuan Luo, Jaw-Town Lin, Emad M El-Omar†, Ming-Shiang Wu†, Jyh-Ming Liou†, for the Taiwan Gastrointestinal Disease and Helicobacter Consortium*

Summary

Background *Helicobacter pylori* infection is an important causal factor of gastric cancer and peptic ulcer disease and is associated with immune thrombocytopenic purpura and functional dyspepsia. In *H pylori* strains, point mutations in the 23S rRNA and *gyrA* genes are associated with clarithromycin resistance and levofloxacin resistance, respectively. Whether the efficacy of molecular testing-guided therapy is non-inferior to that of susceptibility testing-guided therapy for *H pylori* eradication is unclear. Therefore, we aimed to compare the efficacy and safety of molecular testing-guided therapy and traditional culture-based susceptibility testing-guided therapy in first-line and third-line treatment of *H pylori* infection.

Methods We did two multicentre, open-label randomised trials in Taiwan. In trial 1 (done at seven hospitals), treatment-naive individuals infected with *H pylori* who were aged 20 years or older were eligible for study inclusion. In trial 2 (done at six hospitals), individuals aged 20 years or older who failed treatment after two or more eradication therapies for *H pylori* infection were eligible for enrolment. Eligible patients were randomly assigned (1:1) to receive either molecular testing-guided therapy or susceptibility testing-guided therapy. The randomisation sequence was generated by computer using permuted block randomisation with a block size of 4. All investigators were masked to the randomisation sequence. Clarithromycin and levofloxacin resistance were determined by agar dilution test for measuring minimum inhibitory concentrations in the susceptibility testing-guided therapy group, and by PCR and direct sequencing for detection of 23S rRNA and *gyrA* mutations in the molecular testing-guided therapy group. Study participants received clarithromycin sequential therapy, levofloxacin sequential therapy, or bismuth quadruple therapy according to the resistance status to clarithromycin and levofloxacin. The ^{13}C-urease breath test was used to determine the status of *H pylori* infection at least 6 weeks after eradication therapy. The primary outcome was the eradication rate by intention-to-treat analysis. The frequency of adverse effects was analysed in patients with available data. The prespecified margins for non-inferiority were 5% for trial 1 and 10% for trial 2. The trials are ongoing for post-eradication follow-up and registered with ClinicalTrials.gov, NCT03556254 for trial 1, and NCT03555526 for trial 2.

Findings Between March 28, 2018, and April 23, 2021, 560 eligible treatment-naive patients with *H pylori* infection were recruited and randomly assigned to the molecular testing-guided therapy group or the susceptibility testing-guided therapy group in trial 1. Between Dec 28, 2017, and Oct 27, 2020, 320 eligible patients with refractory *H pylori* infection were recruited and randomly assigned to the molecular testing-guided therapy group or the susceptibility testing-guided therapy group in trial 2. 272 men and 288 women were recruited for trial 1, and 98 men and 222 women were recruited for trial 2. In first-line *H pylori* treatment, infection was eradicated in 241 (86%, 95% CI 82–90) of 280 patients in the molecular testing-guided therapy group and 243 (87%, 83–91) of 280 patients in the susceptibility testing-guided therapy group by intention-to-treat analysis (p=0·81). In third-line *H pylori* treatment, infection was eradicated in 141 (88%, 83–93) of 160 patients in the molecular testing-guided therapy group and 139 (87%, 82–92) of 160 patients in the susceptibility testing-guided therapy group by intention-to-treat analysis (p=0·74). The difference in the eradication rate between the molecular testing-guided therapy group and the susceptibility testing-guided therapy group was –0·7% (95% CI –6·4 to 5·0; non-inferiority p=0·071) in trial 1 and 1·3% (–6·0 to 8·5; non-inferiority p=0·0018 in trial 2 by intention-to-treat analysis. We found no difference in adverse effects across both treatment groups in trial 1 and trial 2.

Interpretation Molecular testing-guided therapy was similar to susceptibility testing-guided therapy in first-line therapy and non-inferior to susceptibility testing guided therapy in third-line treatment of *H pylori* infection, supporting the use of molecular testing-guided therapy for *H pylori* eradication.

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*Contributed equally as first author

†Contributed equally as senior and corresponding authors to this work

Division of Gastroenterology and Hepatology, Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan (M-J Chen MD, Chieh-Chang Chen MD, Chien-Chuan Chen MD, Prof J-T Lin MD, Prof M-S Wu MD, Prof J-M Liou MD); **Department of Internal Medicine, National Taiwan University College of Medicine, Taipei, Taiwan** (M-J Chen, Y-J Fang MD, Chieh-Chang Chen, Chien-Chuan Chen, T-H Yang MD, J-Y Lee MD, C-C Yu MD, C-C Kuo MD, M-C Chiu MD, Prof J-T Lin, Prof M-S Wu, Prof J-M Liou); **Integrated Diagnostics and Therapeutics** (M-J Chen) **and Department of Pathology** (Prof C-T Shun MD), **National Taiwan University Hospital, National Taiwan University College of Medicine, Taipei, Taiwan; Division of Gastroenterology and Hepatology, Department of Internal Medicine, Chia-Yi Christ ian Hospital, Chiayi City, Taiwan** (P-Y Chen MD, C-K Chou MD, C-Y Chen MD); **Department of Internal Medicine, National Taiwan University Hospital, Yun-Lin Branch, National Taiwan University College of Medicine, Yun-Lin, Taiwan** (Y-J Fang, T-H Yang, J-Y Lee, C-C Yu, C-C Kuo, M-C Chiu); **Division of Gastroenterology**

and Hepatology, Department of Internal Medicine, Taitung Mackay Memorial Hospital, Taitung, Taiwan (M-J Bair MD); Mackay Medical College, New Taipei, Taiwan (M-J Bair); Department of Internal Medicine, National Taiwan University Hospital, Hsin-Chu Branch, Hsin-Chu, Taiwan (W-H Hu MD, M-H Tsai MD); Department of Internal Medicine, E-DA Hospital and I-Shou University, Kaohsiung City, Taiwan (Y-C Hsu MD, Prof J-T Lin); Department of Pathology, Good Liver Clinic, Taipei, Taiwan (Prof C-T Shun); Healthcare and Services Centre and Division of Gastroenterology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan (Prof J-C Luo MD); UNSW Microbiome Research Centre, St George and Sutherland Clinical Campuses, School of Clinical Medicine, Faculty of Medicine and Health, University of New South Wales, Sydney, NSW, Australia (Prof E M El-Omar MD); Department of Internal Medicine, National Taiwan University Cancer Centre, Taipei, Taiwan (Prof J-M Liou)

Correspondence to:
Prof Jyh-Ming Liou, Department of Internal Medicine, National Taiwan University Hospital, National Taiwan University, College of Medicine, Taipei 100, Taiwan
jyhmingliou@gmail.com

Introduction

Helicobacter pylori infection is an important causal factor of gastric cancer and peptic ulcer disease and is associated with immune thrombocytopenic purpura and functional dyspepsia.[1] Eradication of *H pylori* can reduce the risk of gastric cancer and the recurrence rate of peptic ulcer disease, and relieve the symptoms of functional dyspepsia.[2,3] The efficacy of standard triple therapy has declined to less than 80% in regions with high clarithromycin resistance.[4] A meta-analysis showed that susceptibility testing-guided therapy was superior to empirical therapy in first-line *H pylori* eradication (risk ratio [RR] 1·14, 95% CI 1·08–1·21);[5] however, some trials have shown that empirical bismuth quadruple therapy or concomitant therapy achieved similar eradication rates.[6] For patients with refractory *H pylori* infection, the eradication rate of molecular testing-guided therapy was 78%, compared with 72% in those receiving empirical therapy.[7,8] These contradictory results might be attributable to the differences in the prevalence of antibiotic resistance, the efficacy of empirical therapy, and the strategy of selecting the regimens according to susceptibility testing. Culture-based antimicrobial susceptibility testing is the gold standard method for detection of antibiotic resistance of *H pylori*.[9] However, there are several disadvantages of susceptibility testing, including its time-consuming nature (taking at least 2–3 weeks), variable success rate of culture (75–90%), the difficulty in discontinuing proton pump inhibitors in clinical practice, and the requirement of special conditions for transportation and culture (micro-aerobic). These disadvantages limit the widespread application of susceptibility testing-guided therapy for *H pylori* infection in clinical practice.[10] Culture-independent molecular methods, including PCR or next-generation sequencing (NGS),[11] are more convenient because DNA is stabler and more easily transported than *H pylori* strains. A previous study reported that point mutations at A2143G, A2142G, and A2142C of 23S ribosomal RNA (23S rRNA) were detected in 69·8%, 11·7%, and 2·6% of clarithromycin-resistant *H pylori* strains, respectively.[12,13] Point mutations in 23S rRNA were found to be associated with treatment failure after clarithromycin-based therapy.[12–14] Point mutations at amino acids 87 and 91 of *gyrA* were detected in 90% of levofloxacin resistant *H pylori* strains and were associated with treatment failure after levofloxacin-based therapy.[12] Studies have shown that mutations in PBP1-3, 16S rRNA, *rdxA*, and

Research in context

Evidence before this study

The efficacy of empirical therapy for *Helicobacter pylori* eradication has reduced with the global increase of antibiotic resistance in *H pylori*. Culture-based susceptibility testing-guided therapy was shown to be superior to empirical therapy in first-line treatment of *H pylori* infection. However, culture-based susceptibility testing is time-consuming and inconvenient, which limits its widespread application in clinical practice. Point mutations in 23S ribosomal RNA (23S rRNA) correlate with clarithromycin resistance of *H pylori* and point mutations in *gyrA* correlate with levofloxacin resistance of *H pylori*. However, there is a paucity of direct evidence regarding the efficacy of molecular testing-guided therapy compared with susceptibility testing-guided therapy. We searched PubMed for studies that reported molecular testing-guided therapy in *H pylori* treatment from database inception to Oct 1, 2022, with the search terms "Helicobacter pylori[Title/Abstract])" AND "eradication[Title/Abstract]" AND "genotypic resistance[Title/Abstract]" AND "susceptibility testing[Title/Abstract]"" or "H pylori[Title/Abstr"ct])""AND "eradication[Title/Abst"act]""AND "genotypic[Title/Abst"act]""AND "susceptibility[Title/Abstract]". The article type was limited to "clinical trial" or "randomised controlled trial". Searched articles were not limited to English language publications. Our search confirmed that the two trials reported herein are the first two randomised trials to compare the efficacy and safety of genotypic resistance-guided therapy versus susceptibility testing-guided therapy for *H pylori* eradication.

Added value of this study

In treatment-naive patients, 23S rRNA mutation was detected in 86 (93%) of 92 clarithromycin-resistant strains and *gyrA* mutation was detected in 77 (84%) of 92 levofloxacin-resistant strains. In patients with refractory *H pylori* infection, 23S rRNA mutation was detected in 274 (99%) of 278 clarithromycin-resistant strains and *gyrA* mutation was detected in 197 (94%) of 210 levofloxacin-resistant strains. We showed that the eradication rates of molecular testing-guided therapy and susceptibility testing-guided therapy were similar in first-line treatment (difference –0·7%, 95% CI –6·4 to 5·0), although the difference did not reach the prespecified margin of 5%. Non-inferiority of molecular testing-guided therapy to susceptibility testing-guided therapy of the prespecified margin of 10% was shown in third-line treatment (difference 1·3%, 95% CI –6·0 to 8·5).

Implications of all the available evidence

Molecular testing-guided therapy was similar to susceptibility testing-guided therapy in first-line treatment and not inferior to susceptibility testing-guided therapy in third-line treatment of *H pylori* infection. Both strategies achieved high eradication rates, including in patients with refractory *H pylori* infection. Our results support the use of molecular testing-guided therapy for *H pylori* infection in clinical practice.

frxA were associated with resistance to amoxicillin, tetracycline, and metronidazole, respectively.[13,15]

Whether the efficacy of molecular testing-guided therapy is non-inferior to that of susceptibility testing-guided therapy for *H pylori* eradication remains unclear. Therefore, we did two multicentre, open-label, randomised trials to compare the efficacy and safety of molecular testing-guided therapy and traditional culture-based susceptibility testing-guided therapy in first-line (trial 1) and third-line (trial 2) treatment of *H pylori* infection.

Methods

Study design and participants

Both trial 1 and trial 2 were multicentre, two-arm, parallel assignment, open-label, randomised controlled trials in Taiwan. The study proposals were approved by the institutional review board of each participating hospital. Written informed consent was obtained from all participants before recruitment.

In trial 1 (done at seven hospitals), treatment-naive individuals infected with *H pylori* who were aged 20 years or older were eligible for study inclusion. In trial 2 (done at six hospitals), individuals aged 20 years or older who failed treatment after two or more eradication therapies for *H pylori* infection were eligible for enrolment. Participants were excluded from trials 1 and 2 if they were younger than 20 years, had a history of gastrectomy or gastric malignancy, including adenocarcinoma or lymphoma, had a previous allergic reaction or contraindication to antibiotics (eg, amoxicillin, clarithromycin, levofloxacin, and metronidazole) or bismuth or proton pump inhibitors (esomeprazole), were pregnant or breastfeeding, or had severe concurrent diseases, such as end-stage renal failure or decompensated liver cirrhosis. Study participants were diagnosed with *H pylori* infection at outpatient clinics of each study site and recruited into our study.

Randomisation and masking

Eligible patients were randomly assigned (1:1) to receive either molecular testing-guided therapy or susceptibility testing-guided therapy. Study participants received one of clarithromycin sequential therapy, levofloxacin sequential therapy, or bismuth quadruple therapy based on molecular testing or traditional susceptibility testing for resistance to clarithromycin and levofloxacin. The randomisation sequence was generated by computer using permuted block randomisation with a block size of 4, and participant assignment was concealed in an opaque envelope. All investigators were masked to the randomisation sequence. After obtaining written informed consent from eligible patients, the assignment of treatment regimens was done by contacting an independent research nurse at the National Taiwan University Hospital. The independent research nurse was primarily responsible for keeping the randomisation sequence and handling administrative and coordination tasks related to the trial. Study participants received eradication therapy after the results of molecular testing or minimum inhibitory concentration testing were available.

Procedures

We defined *H pylori* infection as any two positives of ^{13}C-urease breath test (^{13}C-UBT), rapid urease test, histology, or culture, prior to study recruitment. All participants were instructed not to take antibiotics for 4 weeks and not to use proton pump inhibitors for 2 weeks before these tests. After treatment, ^{13}C-UBT was used to detect *H pylori* at least 6 weeks after eradication therapy (appendix p 3).

See Online for appendix

The treatment regimens used in these two trials included 14-day clarithromycin-based sequential therapy containing esomeprazole 40 mg and amoxicillin 1 g twice daily for 7 days, followed by esomeprazole 40 mg, clarithromycin 500 mg, and metronidazole 500 mg twice per day for another 7 days; or 14-day levofloxacin-based sequential therapy containing esomeprazole 40 mg and amoxicillin 1 g twice daily for 7 days, followed by esomeprazole 40 mg, levofloxacin 250 mg and metronidazole 500 mg twice daily for another 7 days; or 10-day bismuth quadruple therapy containing esomeprazole 40 mg twice per day, metronidazole 500 mg three times per day, bismuth tripotassium dicitrate 300 mg (KCB FC tablets; Swiss Pharmaceutical; Tainan City, Taiwan) four times per day, and tetracycline 500 mg four times per day for 10 days. A standardised interview to assess adherence was done by the research nurse at the end of treatment in outpatient clinics at each study site.

The process of gastric biopsy for molecular and minimum inhibitory concentration tests is shown in the appendix (p 3). The clinical practice of molecular testing in this study is described in the appendix (p 19). The minimum inhibitory concentration was determined by agar dilution test, and the breakpoints of clarithromycin (≥1 mg/L), levofloxacin (≥1 mg/L), amoxicillin (≥0·5 mg/L), metronidazole (≥8 mg/L), tetracycline (≥0·5 mg/L), and rifabutin (≥0·5 mg/L) were defined.[12,16] We used the Gentra DNA purification kit (Qiagen; Hilden, Germany) to extract *H pylori* DNA, according to the manufacturer's instructions, from gastric biopsy specimens. After DNA extraction of gastric biopsy specimens, the 23S rRNA fragment and the *gyrA* fragment were amplified by PCR, followed by direct sequencing by an automatic sequencer (ABI PRISM 3100 Genetic Analyzer; Applied Biosystems; Waltham, MA, USA). Point mutations in 23S rRNA (A2142G, A2142C, and A2143G) were defined as 23S rRNA mutations and, similarly, any point mutation that altered the amino acids at position 87, 88, 91, and 97 of *gyrA* genes was defined as a *gyrA* mutation. Detailed methods to detect 23S rRNA and *gyrA* mutations were described in our previous studies.[7,8,12] In trial 1, we first tested for 23S rRNA or clarithromycin resistance, followed by *gyrA* or levofloxacin resistance to determine the treatment regimen. By contrast, in trial 2, *gryA* or levofloxacin

resistance was preferentially used to determine the treatment regimen, followed by 23S rRNA or clartithromycin resistance. Each study participant, whether in the molecular testing-guided therapy group or the susceptibility testing-guided therapy group, underwent both molecular testing and minimum inhibitory concentration testing. Patients had a total of five visits in the study. The first visit was for screening and took place within 1–7 days of enrolment. The second visit involved endoscopy and mucosal biopsy for susceptibility testing, with results available within 1–5 weeks through molecular testing or minimum inhibitory concentration testing. The third visit comprised giving the prescription for eradication therapy, which lasted for 10–14 days. The fourth visit was a post-treatment interview done at the end of the eradication therapy. Finally, the fifth visit involved a post-treatment ^{13}C-UBT 6–8 weeks after eradication, with results available within 1 week.

Outcomes

The primary outcome was the eradication rate by intention-to-treat analysis. The secondary outcomes were the eradication rate by per-protocol analysis and the tolerability and frequency of adverse effects related to *H pylori* treatment. The follow-up for other secondary endpoints is ongoing, including the reinfection rates, long-term changes in the microbiota, and metabolic factors. Common adverse reactions and compliance with medication were assessed at the end of treatment. Sex was collected, and post-hoc stratified analyses were done to explore potential sex-based differences in the study outcomes.

Patients were informed about the common adverse events of the treatment regimens before undergoing eradication therapy. They were requested to keep a record of any symptoms they had during treatment. Adverse events were evaluated by research nurses using a predefined case report form at the end of treatment.

Statistical analysis

We assumed the eradication rate of molecular testing-guided therapy and susceptibility testing-guided therapy to be 98·6% in trial 1 and 88% in trial 2, according to the per-protocol analysis. In trial 1, we estimated a loss to follow-up rate of 4·5% and assumed the eradication rates of both treatments to be 94% in the intention-to-treat analysis. A sample size of at least 280 participants per group (560 in total) was needed to detect a 5% non-inferiority difference at 80% power and a 5% significance level. In trial 2, we assumed a loss to follow-up rate of about 3% and assumed the eradication rates of both treatments to be 85% according to intention-to-treat analysis. A sample size of at least 160 participants in each group (320 in total) was needed to detect a 10% non-inferiority difference at 80% power and a 5% significance level (appendix pp 3–4).

All randomly assigned patients who took study medications were included in the intention-to-treat analysis. According to the study protocols, bismuth quadruple therapy was given when the minimum inhibitory concentration test or molecular tests were unsuccessful. These patients were all included in the intention-to-treat analysis without other statistical interventions. All individuals with protocol violations, such as those who dropped out of the study, who did not take at least 80% of their pills, whose treatment regimen was misclassified, or whose post-treatment *H pylori* status was unknown, were excluded from the per-protocol analysis. Categorical data were compared using the χ^2 test or Fisher's exact test, as appropriate. Continuous data were compared using the student's *t*-test and expressed as mean (SD). P-values were two-tailed, except for non-inferiority, where the significance levels were defined as $p<0·05$.

Under a non-inferiority trial design with a margin of 5% in trial 1 and 10% in trial 2, eradication rates were assessed by intention-to-treat and per-protocol analyses. The null hypothesis (H0) of the randomised trials was that the differences in eradication rates were –5% or less in trial 1 and –10% or less in trial 2. The non-inferiority p-value was calculated in a one-sided test and H0 was rejected if $p<0·05$. We also did a sensitivity analysis using the predicted efficacy of molecular testing-guided therapy and susceptibility testing-guided therapy in regions with different prevalence of clarithromycin and levofloxacin resistance, with efficacy predicted using the methology described by Graham.[17]

Statistical analyses were done using SAS 9.4 for Windows. The two trials were registered with ClinicalTrials.gov, with NCT03556254 for trial 1 and NCT03555526 for trial 2.

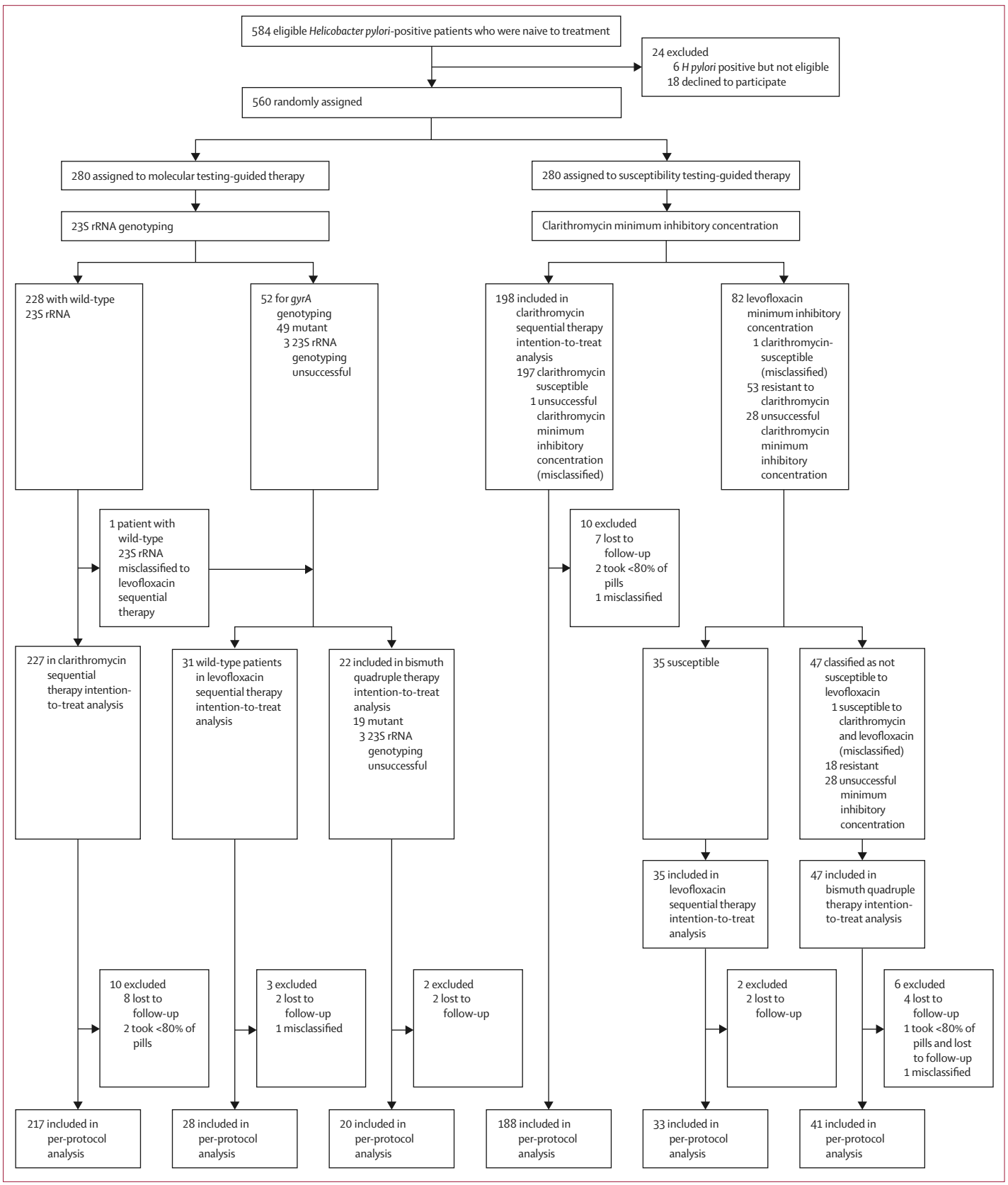
Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between March 28, 2018, and April 23, 2021, 560 eligible treatment-naive patients with *H pylori* infection were recruited and randomly assigned to the molecular testing-guided therapy group or the susceptibility testing-guided therapy group in trial 1 (figure 1; appendix p 4). Between Dec 28, 2017, and Oct 27, 2020, 320 eligible patients with refractory *H pylori* infection were recruited and randomly assigned to the molecular testing-guided therapy group or the susceptibility testing-guided therapy group in trial 2 (figure 2; appendix p 4). Although the study groups had different proportions of clarithromycin resistance, there was no difference in clarithromycin resistance between the molecular testing-guided therapy group and the susceptibility testing-guided therapy group in trial 1 (p=0·148) or trial 2 (p=0·792; appendix p 11).

Figure 1: **Trial profile for trial 1**


584 eligible Helicobacter pylori-positive patients who were naive to treatment
24 excluded
6 H pylori positive but not eligible
18 declined to participate
560 randomly assigned
280 assigned to molecular testing-guided therapy
280 assigned to susceptibility testing-guided therapy
23S rRNA genotyping
Clarithromycin minimum inhibitory concentration
228 with wild-type 23S rRNA
52 for gyrA genotyping
49 mutant
3 23S rRNA genotyping unsuccessful
198 included in clarithromycin sequential therapy intention-to-treat analysis
197 clarithromycin susceptible
1 unsuccessful clarithromycin minimum inhibitory concentration (misclassified)
82 levofloxacin minimum inhibitory concentration
1 clarithromycin-susceptible (misclassified)
53 resistant to clarithromycin
28 unsuccessful clarithromycin minimum inhibitory concentration
1 patient with wild-type 23S rRNA misclassified to levofloxacin sequential therapy
10 excluded
7 lost to follow-up
2 took <80% of pills
1 misclassified
227 in clarithromycin sequential therapy intention-to-treat analysis
31 wild-type patients in levofloxacin sequential therapy intention-to-treat analysis
22 included in bismuth quadruple therapy intention-to-treat analysis
19 mutant
3 23S rRNA genotyping unsuccessful
35 susceptible
47 classified as not susceptible to levofloxacin
1 susceptible to clarithromycin and levofloxacin (misclassified)
18 resistant
28 unsuccessful minimum inhibitory concentration
35 included in levofloxacin sequential therapy intention-to-treat analysis
47 included in bismuth quadruple therapy intention-to-treat analysis
10 excluded
8 lost to follow-up
2 took <80% of pills
3 excluded
2 lost to follow-up
1 misclassified
2 excluded
2 lost to follow-up
2 excluded
2 lost to follow-up
6 excluded
4 lost to follow-up
1 took <80% of pills and lost to follow-up
1 misclassified
217 included in per-protocol analysis
28 included in per-protocol analysis
20 included in per-protocol analysis
188 included in per-protocol analysis
33 included in per-protocol analysis
41 included in per-protocol analysis

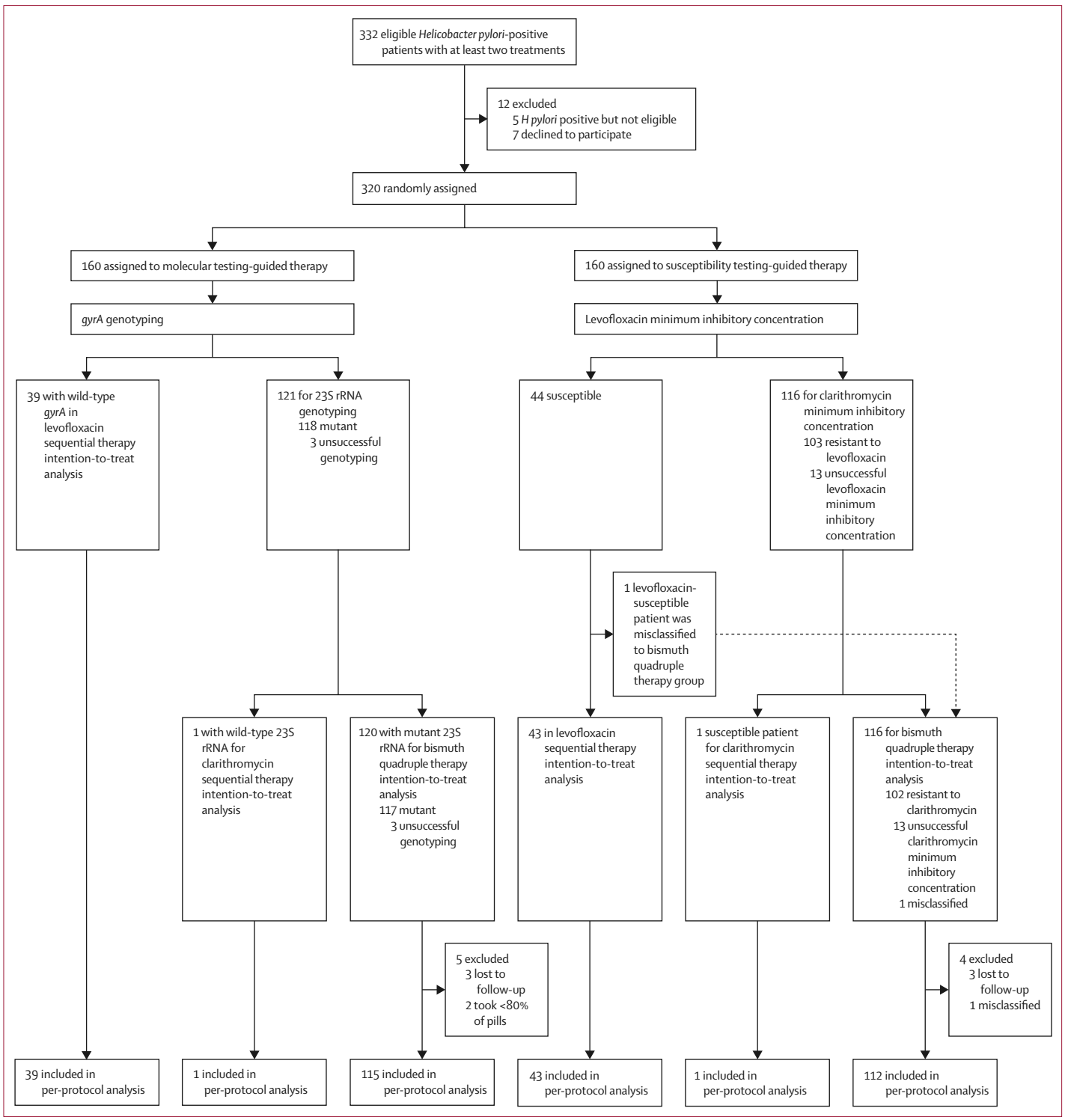


Figure 2: **Trial profile for trial 2**

The mean age was 50·9 years (SD 12·9) in the molecular testing-guided therapy group and 53·4 years (13·6) in the susceptibility testing-guided therapy group in trial 1. The mean age was 54·1 years (11·4) in the molecular testing-guided therapy group and 53·4 years (10·9) in the susceptibility testing-guided therapy group

in trial 2. 272 men and 288 women were recruited for trial 1, and 98 men and 222 women were recruited for trial 2. Demographic data are shown in table 1 and the appendix (pp 12–13).

In trial 1, the success rate of the molecular test (551 [98%] of 560 patients) was higher than that of the antimicrobial susceptibility test (minimum inhibitory concentration test; 494 [88%] of 560 patients). Among the 66 patients with failed minimum inhibitory concentration tests, 26 (39%) had no growth of colony in culture, 34 (52%) had culture contamination and six (9%) had instrument errors. Using minimum inhibitory concentration tests, the prevalence of clarithromycin, levofloxacin, metronidazole, amoxicillin, tetracycline, and rifabutin resistance in treatment-naive patients infected with *H pylori* is shown in table 1, as is the prevalence of 23S rRNA and *gyrA* mutations using molecular tests for gastric biopsy specimens.

In trial 2, the success rate of molecular tests (312 [98%] of 320 patients) was higher than that of antimicrobial susceptibility tests (295 [92%] of 320 patients). Using the minimum inhibitory concentration tests, the prevalence of clarithromycin, levofloxacin, metronidazole, amoxicillin, tetracycline, and rifabutin resistance in patients with refractory *H pylori* infection is shown in table 1, as is the prevalence of 23S rRNA and *gyrA* mutations using molecular tests for gastric biopsy samples.

In first-line *H pylori* treatment in trial 1, infection was eradicated in 241 (86%, 95% CI 82–90) of 280 patients in the molecular testing-guided therapy group and 243 (87%, 83–91) of 280 patients in the susceptibility testing-guided therapy group by intention-to-treat analysis (p=0·81). In the per-protocol analysis, infection was eradicated in 240 (91%, 87–94) of 265 patients in the molecular testing-guided therapy group and 240 (92%, 88–95) of 262 patients in the susceptibility testing-guided therapy group (p=0·68). In third-line *H pylori* treatment in trial 2, infection was eradicated in 141 (88%, 83–93) of 160 patients in the molecular testing-guided therapy group and 139 (87%, 82–92) of 160 patients in the susceptibility testing-guided therapy group by intention-to-treat analysis (p=0·74). In the per-protocol analysis, infection was eradicated in 140 (90%, 86–95) of 155 patients in the molecular testing-guided therapy group and 139 (89%, 84–94) of 156 patients in the susceptibility testing-guided therapy group (p=0·72; table 2; figure 3).

Using the non-inferiority margin of 5% in trial 1, the difference in the eradication rate between the molecular testing-guided therapy group and the susceptibility testing-guided therapy group was –0·7% (95% CI –6·4 to 5·0; non-inferiority p=0·071) according to the intention-to-treat analysis and was –1·0% (–5·9 to 3·8; non-inferiority p=0·059) according to the per-protocol analysis (table 2; figure 3). Using the prespecified non-inferiority margin of 10% in trial 2, the difference in the eradication rate between the molecular testing-guided therapy group and the susceptibility testing-guided therapy group was 1·3% (–6·0 to 8·5; non-inferiority p=0·0018) in the intention-to-treat analysis and 1·2% (–5·5 to 8·0; non-inferiority p=0·0012) in the per-protocol analysis. In third-line treatment, the efficacy in the molecular testing-guided therapy group was not inferior to the efficacy in the susceptibility testing-guided therapy group (table 2; figure 3). Meta-analysis of the two trials showed that the pooled estimates of risk difference between the molecular testing-guided therapy group and the susceptibility testing-guided therapy group was –0·03% (–4·4 to 4·5) according to the intention-to-treat analysis and –0·3% (–4·2 to 3·7) according to the per-protocol analysis (appendix p 5).

We found no significant differences in the frequency of adverse effects between the molecular testing-guided therapy group and the susceptibility testing-guided therapy group in trial 1 and trial 2 (appendix p 14). The frequency of any adverse reactions (p=0·0063 in trial 1 and p=0·0042 in trial 2), dizziness (p=0·049 in trial 1), nausea (p=0·0006 in trial 1 and p=0·0008 in trial 2), and vomiting (p=0·0004 in trial 1 and p=0·031 in trial 2) were more common in

	Trial 1 (first-line)		Trial 2 (third-line)	
	Molecular testing-guided therapy (n=280)	Susceptibility testing-guided therapy (n=280)	Molecular testing-guided therapy (n=160)	Susceptibility testing-guided therapy (n=160)
Age, years	50·9 (12·9)	53·4 (13·6)	54·1 (11·4)	53·4 (10·9)
Sex				
Male	131 (47%)	141 (50%)	48 (30%)	50 (31%)
Female	149 (53%)	139 (50%)	112 (70%)	110 (69%)
Cigarette smoking	35/278 (13%)	62 (22%)	21/159 (13%)	24/155 (15%)
Alcohol drinking	69/279 (25%)	64 (23%)	40/159 (25%)	54/155 (49%)
Oesophagogastroduodenoscopy finding				
Barrett's oesophagus	1 (<1%)	1 (<1%)	0	1 (1%)
Gastritis	131 (47%)	121 (43%)	119 (74%)	119 (74%)
Duodenal ulcer	66 (24%)	57 (20%)	7 (4%)	15 (9%)
Duodenal ulcer scar	56 (20%)	64 (16%)	20 (13%)	17 (11%)
Gastric ulcer	72 (26%)	77 (28%)	15 (9%)	21 (13%)
Gastric ulcer scar	8 (3%)	10 (4%)	2 (1%)	3 (2%)
Intestinal metaplasia	13 (5%)	18 (6%)	7 (4%)	7 (4%)
Atrophic gastritis	12 (4%)	19 (7%)	3 (2%)	4 (3%)
Antimicrobial resistance				
Clarithromycin resistance	39/243 (16%)	53/251 (21%)	140/148 (95%)	138/147 (94%)
Levofloxacin resistance	51/243 (21%)	45/251 (18%)	112/148 (76%)	103/147 (70%)
Metronidazole resistance	54/243 (22%)	64/251 (25%)	100/148 (68%)	106/147 (72%)
Amoxicillin resistance	7/243 (3%)	4/251 (2%)	24/148 (16%)	35/147 (24%)
Tetracycline resistance	13/243 (5%)	15/251 (6%)	14/148 (9%)	16/147 (11%)
Rifabutin resistance	0	1/251 (<1%)	0	1/147 (1%)
23S rRNA mutation (tissue)	49/277 (18%)	63/276 (23%)	150 (94%)	151/159 (95%)
gyrA mutation (tissue)	55/276 (20%)	52/275 (19%)	118/157 (75%)	103/155 (66%)
23S rRNA mutation (strain)	36/247 (15%)	54/253 (21%)	139/148 (94%)	139/149 (93%)
gyrA mutation (strain)	33/240 (14%)	38/251 (15%)	107/148 (72%)	98/149 (66%)

Data are mean (SD), n (%), or n/N (%).

Table 1: **Demographic characteristics and prevalence of antimicrobial resistance of participants receiving molecular testing-guided therapy or susceptibility testing-guided therapy**

	Trial 1 (first-line)				Trial 2 (third-line)			
	Molecular testing-guided therapy (n=280)	Susceptibility testing-guided therapy (n=280)	p-value*	Difference between molecular testing-guided therapy and susceptibility testing-guided therapy	Molecular testing-guided therapy (n=160)	Susceptibility testing-guided therapy (n=160)	p-value*	Difference between molecular testing-guided therapy and susceptibility testing-guided therapy
Eradication rates								
Intention-to-treat analysis	241 (86%)	243 (87%)	0·81	−0·7%, 95% CI −6·4 to 5·0; p=0·071	141 (88%)	139 (87%)	0·74	1·3%, 95% CI −6·0 to 8·5; p=0·0018
Per-protocol analysis	240/265 (91%)	240/262 (92%)	0·68	−1·0%, 95% CI −5·9 to 3·8; p=0·059	140/155 (90%)	139/156 (89%)	0·72	1·2%, 95% CI −5·5 to 8·0; p=0·0012
Subgroup analyses								
Sex								
Female	132/149 (89%)	122/139 (88%)	0·83	0·8%, 95% CI −6·6 to 8·3; p=0·065	100/112 (89%)	98/110 (89%)	0·96	0·2%, 95% CI −8·0 to 8·4; p=0·010
Male	109/131 (83%)	121/141 (86%)	0·55	−2·6%, 95% CI −11·2 to 6·0; p=0·29	41/48 (85%)	41/50 (82%)	0·65	3·4%, 95% CI −11·2 to 18·0; p=0·041
Regimen (intention to treat)								
Clarithromycin sequential therapy	196/227 (86%)	174/198 (88%)	0·64	−1·5%, 95% CI −7·9 to 4·8; p=0·14	0†	0†	..	..
Levofloxacin sequential therapy	27/31 (87%)	30/35 (86%)	1·0	1·4%, 95% CI −15·2 to 17·9; p=0·23	37/39 (95%)†	41/43 (95%)†	1·0	−0·5%, 95% CI −9·8 to 8·9; p=0·055
Bismuth quadruple therapy	18/22 (82%)	39/47 (83%)	1·0	−1·2%, 95% CI −20·5 to 18·2; p=0·35	104/120 (87%)†	98/116 (85%)†	0·63	2·2%, 95% CI −6·8 to 11·2; p=0·0048
Study sites (intention to treat)								
National Taiwan University Hospital	59/64 (92%)‡	63/67 (94%)‡	0·74	−1·8%, 95% CI −10·5 to 6·8; p=0·25	120/137 (88%)	108/126 (86%)	0·65	1·9%, 95% CI −6·4 to 10·1; p=0·0030
National Taiwan University YunLin branch	87/91 (96%)‡	75/80 (94%)‡	0·74	1·9%, 95% CI −4·9 to 8·6; p=0·031	..	..	..	..
Chia-Yi Christian Hospital	60/84 (71%)‡	69/87 (79%)‡	0·23	−7·9%, 95% CI −20·8 to 5·0; p=0·67	..	..	..	..
Other§	35/41 (85%)‡	36/46 (78%)‡	0·39	7·1%, 95% CI −9·0 to 23·2; p=0·076	21/23 (91%)	31/34 (91%)	1·0	0·1%, 95% CI −14·8 to 15·1; p=0·13

Data are n (%) or n/N (%), unless otherwise indicated. *p values for the comparison between categorical data using the χ^2 test or Fisher's exact test, as appropriate, and a two-tailed p<0·05 was defined as significant. †There were statistical differences in eradication rates across different regimens in trial 2: p=0·0093 in the molecular testing-guided therapy group and p=0·0071 in the susceptibility testing-guided therapy group. ‡There were statistical differences in eradication rates across different study sites in trial 1: p<0·0001 in the molecular testing-guided therapy group and p=0·0035 in the susceptibility testing-guided therapy group. §Other hospitals in trial 1 included National Taiwan University HsinChu branch, China Medical University Hospital, National Cheng Kung University Hospital, and Taitung Mackay Memorial Hospital; other hospitals in trial 2 included National Taiwan University YunLin branch, National Taiwan University HsinChu branch, Chia-Yi Christian Hospital, Taipei Veterans General Hospital, and Taitung Mackay Memorial Hospital.

Table 2: **Comparisons of the eradication rates and adverse effects between the molecular testing-guided therapy and susceptibility testing-guided therapy groups**

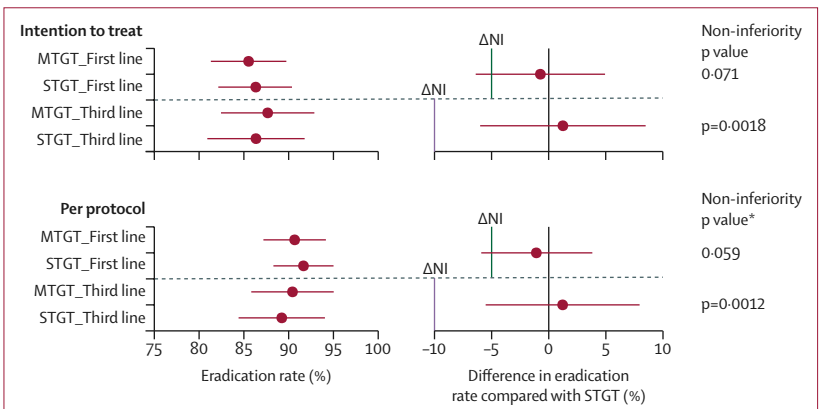


Figure 3: **Eradication rates of each therapy group**
Error bars are 95% CIs. ΔNI=non-inferiority margin. MTGT=molecular testing-guided therapy. STGT=susceptibility testing-guided therapy.

patients who received bismuth quadruple therapy than in those who received clarithromycin or levofloxacin regimens. Additionally, diarrhoea occurred most frequently in patients who received clarithromycin sequential therapy (p=0·0073 in trial 1; appendix p 15). Most study participants took medication regularly and took at least 80% of pills. Few patients discontinued the study drugs due to adverse effects (appendix p 14).

We found no significant differences in the eradication rates between men (p=0·83 in trial 1 and 0·96 in trial 2) and women (p=0·55 in trial 1 and 0·65 in trial 2). We found no significant differences in the eradication rate of clarithromycin sequential therapy between the molecular testing-guided therapy group and the susceptibility testing-guided therapy group in the intention-to-treat analysis (p=0·64) in trial 1 (table 2). Similarly, there were no significant differences in the eradication rates of levofloxacin sequential therapy or bismuth quadruple therapy between the molecular testing-guided therapy

	Trial 1 (first-line)			Trial 2 (third-line)		
	Susceptible	Resistant	κ coefficient for susceptible *vs* resistant; p value	Susceptible	Resistant	κ coefficient for susceptible *vs* resistant; p value
Clarithromycin resistance in 23S rRNA (tissue), minimum inhibitory concentration						
Wild-type	382	6	0·86 (95% CI 0·80–0·92); p<0·0001	14	4	0·79 (95% CI 0·63–0·94); p<0·0001
Mutant	16	86	..	3	274	..
Levofloxacin resistance in *gyrA* (tissue), minimum inhibitory concentration						
Wild-type	374	19	0·76 (95% CI 0·69–0·83); p<0·0001	73	13	0·84 (95% CI 0·77–0·91); p<0·0001
Mutant	18	77	..	6	197	..

Table 3: **Correlation between susceptibility testing and molecular testing determined from tissue biopsies**

group and the susceptibility testing-guided therapy group in intention-to-treat analyses in trial 1 and trial 2 (table 2). We observed a lower eradication rate in the molecular testing-guided therapy group (60 [71%] of 84 patients) and the susceptibility testing-guided therapy group (69 [79%] of 87 patients) at one study site (Chia-Yi Christian Hospital) compared with the other study sites in trial 1 (table 2). The eradication rates in the molecular testing-guided therapy and susceptibility testing-guided therapy groups were higher in the National Taiwan University Hospital and the National Taiwan University YunLin branch compared with the other study sites, according to intention-to-treat analysis (table 2). However, there were no significant differences in the eradication rate between the molecular testing-guided therapy group and susceptibility testing-guided therapy group at different study sites in both trial 1 and trial 2 (table 2). Demographic characteristics of participants at different study sites and the eradication rates for intention-to-treat and per-protocol analyses are shown in the appendix (pp 16–17). We observed a lower compliance with study drugs and a higher loss to follow-up at Chia-Yi Christian Hospital compared with the other study sites (appendix pp 16–17).

The effects of phenotypic resistance on the eradication efficacy of different treatment regimens against *H pylori* are summarised in the appendix (p 18). The distribution of the minimum inhibitory concentrations for all tested antibiotics in trial 1 and trial 2 are shown in the appendix (pp 6–7). 23S rRNA mutation detected using gastric biopsy specimens were associated with clarithromycin resistance in trial 1 (κ coefficient 0·86, 95% CI 0·80–0·92; p<0·0001) and trial 2 (κ coefficient 0·79, 0·63–0·94; p<0·0001; table 3). In trial 1, 23S rRNA mutation was detected in 86 (93%) of 92 clarithromycin-resistant strains (table 3), and 97 (95%) of 102 mutations were detected at base pair 2143 (table 4). In trial 2, 23S rRNA mutation was detected in 274 (99%) of 278 clarithromycin-resistant strains (table 3), and 272 (98%) of 277 mutations were detected at base pair 2143 (table 4). The minimum inhibitory concentration value at which growth was inhibited in 50% of isolates (MIC_{50}) of 23S rRNA mutations at 2142 and 2143 was 8 and 32, respectively, whereas the MIC_{50} for wild-type 23S rRNA was 0·03 in trial 1. The MIC_{50} of 23S rRNA mutations at 2142 and 2143 was 128 and 32, respectively, whereas the MIC_{50} for wild-type 23S rRNA was 0·06 in trial 2 (table 4).

	Trial 1 (first-line)			Trial 2 (third-line)		
	N	MIC_{50}	MIC_{90}	N	MIC_{50}	MIC_{90}
Clarithromycin for 23S rRNA (biopsy)						
Wild-type	388	0·03	0·06	18	0·06	64
Mutant	102	32	64	277	32	64
Base pair 2142 mutant	6	8	>64	5	128	128
Base pair 2143 mutant	97	32	64	272	32	64
Levofloxacin for *gyrA* (biopsy)						
Wild-type	393	0·5	0·5	86	0·5	2
Mutant	95	4	16	203	8	32
Codon 87 mutant	48	8	32	141	8	32
Codon 88 mutant	4	1·5	2	1	2	2
Codon 91 mutant	47	4	16	77	8	32
Codon 97 mutant	6	0·5	1	0	..	..

MIC_{50}=minimum inhibitory concentration value at which growth was inhibited in 50% of isolates. MIC_{90}=minimum inhibitory concentration value at which growth was inhibited in 90% of isolates.

Table 4: **Minimum inhibitory concentration values for clarithromycin and levofloxacin mutations**

GyrA mutation detected using gastric biopsy specimens were associated with levofloxacin resistance in trial 1 (κ coefficient 0·76, 95% CI 0·69–0·83; p<0·0001) and trial 2 (κ coefficient 0·84, 0·77–0·91; p<0·0001). In trial 1, *gyrA* mutation was detected in 77 (84%) of 92 levofloxacin-resistant strains (table 3), and 48 (51%) of 95 mutations were detected at codon 87, four (4%) were detected at codon 88, 47 (49%) were detected at codon 91, and six (6%) were detected at codon 97 (table 4). In trial 2, *gyrA* mutation was detected in 197 (94%) of 210 levofloxacin-resistant strains (table 3), and 141 (69%) of 203 mutations were detected at codon 87, one (<1%) was detected at codon 88, 77 (38%) were detected at codon 91, and no mutations were detected at codon 97 (table 4). The MIC_{50} of *gyrA* mutations at codon 87 was 8, at codon 88 was 1·5, at codon 91 was 4, and at codon 97 was 0·5, whereas the MIC_{50} for wild-type *gyrA* was 0·5 in trial 1. The MIC_{50} of *gyrA* mutations at codon 87 was 8, at codon 88 was 2, and at codon 91 was 8, whereas the MIC_{50} for wild-type *gyrA* was

0·5 in trial 2 (table 4). The 2143 base pair mutation is dominant among 23S rRNA mutations, and point mutations at codon 87 and codon 91 were dominant among *gyrA* mutations.

In a sensitivity analysis using predicted efficacy, we showed that molecular testing-guided therapy and susceptibility testing-guided therapy had similar eradication rates in regions with different prevalence of clarithromycin and levofloxacin resistance (appendix pp 8–10). Additionally, previous use of antibiotics for *H pylori* eradication by study participants in trial 2 is shown in the appendix (p 20).

Discussion

To our knowledge, this is the first study to compare molecular testing-guided therapy with susceptibility testing-guided therapy in first-line and third-line treatment of *H pylori* infection. Point mutations at 23S rRNA and *gyrA* were associated with phenotypic clarithromycin and levofloxacin resistance, respectively. Our results lend support to the use of culture-free molecular testing-guided therapy for *H pylori* eradication.

Traditional culture-based susceptibility testing-guided therapy has been recommended to guide therapy for refractory *H pylori* infection and resistant *Mycobacterium tuberculosis*.[9,18–20] The challenge of successful culture of *H pylori* is that it requires culture under microaerophilic conditions and specific culture agars, takes 7–10 days or longer to grow, and requires staff with specific training to grow and identify strains. Molecular testing is a promising approach to identify infectious diseases and to detect antimicrobial resistance. Molecular testing has several advantages over susceptibility testing (minimum inhibitory concentration). For example, molecular testing does not require *H pylori* culture, is less time consuming, and intragastric juice or stool specimens can be used to detect genotypic resistance directly. Genotypic antimicrobial resistance detected by next-generation sequencing is associated with phenotypic resistance for *M tuberculosis*, but the availability of such novel technology remains low for tuberculosis in clinical practice.[19]

The Maastricht consensus report for the management of *H pylori* infection is followed in Taiwan. Clarithromycin-based therapy or bismuth quadruple therapy are recommended in first-line treatment, whereas levofloxacin-based therapy or bismuth quadruple therapy are recommended in second-line treatment. If susceptibility tests are unavailable, bismuth quadruple therapy would be the empirical first-line treatment of choice in regions with high clarithromycin resistance. The complexity of prescription use is a problem for sequential therapy. Sequential therapy was used in this study because our previous studies showed excellent eradication rates (97–99%) in clarithromycin-susceptible or levofloxacin-susceptible strains.[14,21,22] Besides, compliance with sequential therapy was high in this study. Nevertheless, concomitant therapy is the preferred empirical non-bismuth quadruple therapy when susceptibility testing is not done.

The prevalence of amoxicillin resistance in patients with refractory *H pylori* infection in Taiwan was previously reported to be around 12–13%,[7] and our study found a resistance rate of more than 20%. This finding could be attributed to previous amoxicillin use, although not all patients had complete medication histories. In trial 2, about 93% of patients received clarithromycin-containing regimens and 70% of patients received levofloxacin-containing regimens in their previous therapy. Higher proportions of participants receiving clarithromycin-containing and levofloxacin-containing regimens are associated with higher prevalence of clarithromycin and levofloxacin resistance, highlighting the advantage of susceptibility or molecular testing, which can assign these patients to appropriate treatment regimens.

Our trial results can also be generalised to countries that use bismuth quadruple therapy as a first-line treatment. Patients can be assigned levofloxacin-based or clarithromycin-based regimens according to their resistance to these two antibiotics. Rifabutin-based therapy can be used for patients with dual resistance to levofloxacin and clarithromycin. We constructed *H pylori* normograms[17] (in a sensitivity analysis using predicted efficacy of therapy) to assess the eradication rate of molecular testing-guided therapy and susceptibility testing-guided therapy in regions with different prevalence of levofloxacin and clarithromycin resistance. We showed similar efficacy of molecular testing-guided therapy and susceptibility testing-guided therapy in regions with low-to-moderate resistance of levofloxacin and clarithromycin in first-line treatment and very high resistance in third-line treatment. Trial 1 and trial 2 provided direct evidence of the similar efficacies of molecular testing-guided therapy and susceptibility testing-guided therapy in populations with varying levels of antibiotic resistance against *H pylori*.

Several commercial kits are now available for detection of clarithromycin (A2146C, 2146G, and A2147G) and fluoroquinolone (N87K at position 87; D91N, D91G, D91Y at position 91 in the *gyrA* gene) resistance for *H pylori*.[23,24] A meta-analysis reported that the pooled sensitivity and specificity of the A2142G/C and/or A2143G combination in biopsy specimens for detection of clarithromycin resistance was 96% and 96%, respectively, and the pooled sensitivity and specificity of the *gyrA* gene mutations for detection of quinolone resistance was 97% and 99%, respectively, indicating that molecular methods are reliable for the detection of clarithromycin and fluoroquinolone resistance by *H pylori*.[18] Point mutations in 23S rRNA and *gyrA* were also associated with treatment failure after clarithromycin-based and levofloxacin-based therapies, respectively.[12,25–28] A2143G and A2142G mutations detected by next-generation sequencing have been found to be associated with treatment failure, with mutations detected

in 14 (88%) of 16 patients with treatment failure versus only in four (10%) of 42 patients with treatment success.[29] The results of this study provide support for the application of molecular testing-guided therapy for *H pylori* eradication in daily clinical practice.

However, eradication rates in our per-protocol analysis were only around 90% in first-line therapy, even under the guidance of antibiotic resistance and optimised regimen use. Heteroresistance of *H pylori* isolates—which involved a mixture of susceptible and resistant patterns—could be an explanation. A meta-analysis showed that the prevalence of heteroresistance to clarithromycin and metronidazole in *H pylori*-positive samples was approximately 7% and 14%, respectively.[30] Fluorescence in situ hybridisation showed that nearly half of clarithromycin-resistant *H pylori* strains were heteroresistant cases.[31,32] Another meta-analysis showed that the prevalence of heteroresistance of *H pylori* to clarithromycin, levofloxacin, metronidazole, amoxicillin, and tetracycline was 60·1%, 46·1%, 61·1%, 3·8%, and 21·1%, respectively.[33] Another explanation could be that the accuracy of various diagnostic tests, including the antimicrobial susceptibility testing, is not 100%, resulting in misclassification of antibiotic resistance. We also observed a lower compliance with study drugs and a higher loss to follow-up at Chia-Yi Christian Hospital compared with the other study sites, which might partly explain why the eradication rate in Chia-Yi Christian Hospital was lower than at other study sites. Overall, the eradication rates in the molecular testing-guided therapy group and the susceptibility testing-guided therapy group were over 90% in third-line treatment, which is higher than the 78% eradication rate reported in our previous study of refractory *H pylori* infection.[7] The use of bismuth quadruple therapy in the present study rather than tetracycline sequential therapy might explain the higher eradication rate.[7]

The strengths of this study include the large sample size, separate trials for treatment-naive (trial 1) and refractory patients (trial 2), the high success rates of *H pylori* culture and susceptibility testing and detection of genotypic resistance using gastric biopsy specimens, and the high eradication rate in third-line therapy. Nevertheless, the study has some limitations. First, first-line therapy did not achieve the expected high eradication rate, which could be attributed to the aforementioned reasons; furthermore, the COVID-19 pandemic led to a high dropout rate among treatment-naive patients. Second, the therapy in this trial was not guided by susceptibility testing for amoxicillin and tetracycline because of low primary resistance rates. The molecular resistance mechanisms of amoxicillin, tetracycline, and metronidazole in relation to *H pylori* are more complex, and associations with treatment failure remain controversial. Third, gastric biopsy specimens were used for molecular testing in this study. Further studies are needed to assess whether the use of stool samples can achieve similarly high accuracy and eradication rates.

In conclusion, we showed that genotypic resistance detected by molecular methods was similar to susceptibility testing-guided therapy in first-line treatment and non-inferior to susceptibility testing-guided therapy in third-line treatment of *H pylori* infection. Both strategies achieved high eradication rates, including in those with refractory *H pylori* infection. Our results support the use of molecular testing-guided therapy for *H pylori* infection in clinical practice. Whether susceptibility testing-guided therapy is superior to empirical therapy, particularly in third-line treatment, remains controversial and warrants further, large scale, randomised trials.

Contributors

M-JC and J-ML conceived and designed the study. All authors collected the study data. M-JC and J-ML analysed the results. M-JC, P-YC, EME-O, M-SW, and J-ML interpreted the results. M-JC and J-ML drafted the article and all authors commented on drafts and approved the final version of the article, including the authorship list. All authors had full access to all the data in the study and accept responsibility for the decision to submit for publication. M-JC and J-ML have directly accessed and verified the underlying data reported in the manuscript.

Declaration of interests

We declare no competing interests.

Data sharing

Individual deidentified participant data can be shared for collaborative research proposals after the publication of the primary study results and key secondary manuscripts (around 3 years after the publication of the primary results). Applicants will be expected to meet the costs associated with the preparation of data or statistical analysis. Applications and additional requests for information should be made to the Taiwan Gastrointestinal Disease and Helicobacter Consortium via Jyh-Ming Liou (jyhmingliou@gmail.com). Approval will depend on the scientific value of the proposal, compatibility with the original patient consent, and data protection legislation. Subject to approval, data will be shared via the secure online storage infrastructure of the National Taiwan University Hospital, in accordance with the regulations of Taiwan.

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References

1 Malfertheiner P, Megraud F, Rokkas T, et al. Management of *Helicobacter pylori* infection: the Maastricht VI/Florence consensus report. *Gut* 2022; **71:** 1724–62.

2 Liou JM, Malfertheiner P, Lee YC, et al. Screening and eradication of *Helicobacter pylori* for gastric cancer prevention: the Taipei global consensus. *Gut* 2020; **69:** 2093–112.

3 Katelaris P, Hunt R, Bazzoli F, et al. *Helicobacter pylori* World Gastroenterology Organization Global Guideline. *J Clin Gastroenterol* 2023; **57:** 111–26.

4 Graham DY, Hernaez R, Rokkas T. Cross-roads for meta-analysis and network meta-analysis of *H pylori* therapy. *Gut* 2022; **71:** 643–50.

5 Ma Q, Li H, Liao J, Cai Z, Zhang B. Tailored therapy for *Helicobacter pylori* eradication: a systematic review and meta-analysis. *Front Pharmacol* 2022; **13:** 908202.

6 Nyssen OP, Espada M, Gisbert JP. Empirical *vs* susceptibility-guided treatment of *Helicobacter pylori* infection: a systematic review and meta-analysis. *Front Microbiol* 2022; **13:** 913436.

7 Liou JM, Chen PY, Luo JC, et al. Efficacies of genotypic resistance-guided *vs* empirical therapy for refractory *Helicobacter pylori* infection. *Gastroenterology* 2018; **155:** 1109–19.

8 Liou JM, Chen CC, Chang CY, et al. Efficacy of genotypic resistance-guided sequential therapy in the third-line treatment of refractory *Helicobacter pylori* infection: a multicentre clinical trial. *J Antimicrob Chemother* 2013; **68:** 450–56.

9 Mégraud F, Lehours P. *Helicobacter pylori* detection and antimicrobial susceptibility testing. *Clin Microbiol Rev* 2007; **20:** 280–322.

10 Gisbert JP. "Rescue" regimens after *Helicobacter pylori* treatment failure. *World J Gastroenterol* 2008; **14:** 5385–402.

11 Graham DY, Moss SF. Antimicrobial susceptibility testing for *Helicobacter pylori* is now widely available: when, how, why. *Am J Gastroenterol* 2022; **117:** 524–28.

12 Liou JM, Chang CY, Sheng WH, et al. Genotypic resistance in *Helicobacter pylori* strains correlates with susceptibility test and treatment outcomes after levofloxacin- and clarithromycin-based therapies. *Antimicrob Agents Chemother* 2011; **55:** 1123–29.

13 Mégraud F. *H pylori* antibiotic resistance: prevalence, importance, and advances in testing. *Gut* 2004; **53:** 1374–84.

14 Liou JM, Chen CC, Chen MJ, et al. Sequential versus triple therapy for the first-line treatment of *Helicobacter pylori*: a multicentre, open-label, randomised trial. *Lancet* 2013; **381:** 205–13.

15 Tshibangu-Kabamba E, Yamaoka Y. *Helicobacter pylori* infection and antibiotic resistance—from biology to clinical implications. *Nat Rev Gastroenterol Hepatol* 2021; **18:** 613–29.

16 Chen MJ, Wu MS, Chen CC, et al. Impact of amoxicillin resistance on the efficacy of amoxicillin-containing regimens for *Helicobacter pylori* eradication: analysis of five randomized trials. *J Antimicrob Chemother* 2017; **72:** 3481–89.

17 Wang YH, Li Z, Wang L, et al. A systematic review and meta-analysis of genotypic methods for detecting antibiotic resistance in *Helicobacter pylori*. *Helicobacter* 2018; **23:** e12467.

18 Lange C, Aarnoutse R, Chesov D, et al. Perspective for precision medicine for tuberculosis. *Front Immunol* 2020; **11:** 566608.

19 Walker TM, Miotto P, Köser CU, et al. The 2021 WHO catalogue of *Mycobacterium tuberculosis* complex mutations associated with drug resistance: a genotypic analysis. *Lancet Microbe* 2022; **3:** e265–73.

20 Liou JM, Chen CC, Fang YJ, et al. 14 day sequential therapy versus 10 day bismuth quadruple therapy containing high-dose esomeprazole in the first-line and second-line treatment of *Helicobacter pylori*: a multicentre, non-inferiority, randomized trial. *J Antimicrob Chemother* 2018; **73:** 2510–18.

21 Liou JM, Jiang XT, Chen CC, et al. Second-line levofloxacin-based quadruple therapy versus bismuth-based quadruple therapy for *Helicobacter pylori* eradication and long-term changes to the gut microbiota and antibiotic resistome: a multicentre, open-label, randomised controlled trial. *Lancet Gastroenterol Hepatol* 2023; **8:** 228–41.

22 Graham DY. Hp-normogram (normo-graham) for assessing the outcome of *H pylori* therapy: effect of resistance, duration, and CYP2C19 genotype. *Helicobacter* 2016; **21:** 85–90.

23 Mégraud F, Bénéjat L, Ontsira Ngoyi EN, Lehours P. Molecular approaches to identify *Helicobacter pylori* antimicrobial resistance. *Gastroenterol Clin North Am* 2015; **44:** 577–96.

24 Ierardi E, Giorgio F, Iannone A, et al. Noninvasive molecular analysis of *Helicobacter pylori*: is it time for tailored first-line therapy? *World J Gastroenterol* 2017; **23:** 2453–58.

25 De Francesco V, Margiotta M, Zullo A, et al. Clarithromycin-resistant genotypes and eradication of *Helicobacter pylori*. *Ann Intern Med* 2006; **144:** 94–100.

26 Lee HJ, Kim JI, Cheung DY, et al. Eradication of *Helicobacter pylori* according to 23S ribosomal RNA point mutations associated with clarithromycin resistance. *J Infect Dis* 2013; **208:** 1123–30.

27 Seo SI, Do BJ, Kang JG, et al. *Helicobacter pylori* eradication according to sequencing-based 23S ribosomal RNA point mutation associated with clarithromycin resistance. *J Clin Med* 2019; **9:** 54.

28 Ong S, Kim SE, Kim JH, et al. *Helicobacter pylori* eradication rates with concomitant and tailored therapy based on 23S rRNA point mutation: a multicenter randomized controlled trial. *Helicobacter* 2019; **24:** e12654.

29 Nezami BG, Jani M, Alouani D, Rhoads DD, Sadri N. *Helicobacter pylori* mutations detected by next-generation sequencing in formalin-fixed, paraffin-embedded gastric biopsy specimens are associated with treatment failure. *J Clin Microbiol* 2019; **57:** e01834-18.

30 Kouhsari E, Sadeghifard N, Khadiv A, et al. Heteroresistance to clarithromycin and metronidazole in patients with a *Helicobacter pylori* infection: a systematic review and meta-analysis. *Ann Clin Microbiol Antimicrob* 2022; **21:** 19.

31 Kocsmár É, Kocsmár I, Buzás GM, et al. *Helicobacter pylori* heteroresistance to clarithromycin in adults—new data by in situ detection and improved concept. *Helicobacter* 2020; **25:** e12670.

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

33 Keikha M, Karbalaei M. Prevalence of antibiotic heteroresistance associated with *Helicobacter pylori* infection: a systematic review and meta-analysis. *Microb Pathog* 2022; **170:** 105720.