Long-term changes of gut microbiota, antibiotic resistance, and metabolic parameters after *Helicobacter pylori* eradication: a multicentre, open-label, randomised trial

Jyh-Ming Liou, Chieh-Chang Chen, Chih-Min Chang*, Yu-Jen Fang*, Ming-Jong Bair, Po-Yueh Chen, Chi-Yang Chang, Yao-Chun Hsu, Mei-Jyh Chen, Chien-Chuan Chen, Ji-Yuh Lee, Tsung-Hua Yang, Jiing-Chyuan Luo, Chi-Yi Chen, Wen-Feng Hsu, Yen-Nien Chen, Jeng-Yih Wu, Jaw-Town Lin, Tzu-Pin Lu, Eric Y Chuang†, Emad M El-Omar†, Ming-Shiang Wu†, for the Taiwan Gastrointestinal Disease and Helicobacter Consortium*

Summary

Background In first-line treatment of *Helicobacter pylori*, we have previously shown that the eradication frequency was 83·7% (95% CI 80·4–86·6) for triple therapy for 14 days (T14; lansoprazole 30 mg, amoxicillin 1 g, and clarithromycin 500 mg, all given twice daily), 85·9% (82·7–88·6) for concomitant therapy for 10 days (C10; lansoprazole 30 mg, amoxicillin 1 g, clarithromycin 500 mg, and metronidazole 500 mg, all given twice daily), and 90·4% (87·6–92·6) for bismuth quadruple therapy for 10 days (BQ10; bismuth tripotassium dicitrate 300 mg four times a day, lansoprazole 30 mg twice daily, tetracycline 500 mg four times a day, and metronidazole 500 mg three times a day). In this follow-up study, we assess short-term and long-term effects of these therapies on the gut microbiota, antibiotic resistance, and metabolic parameters.

Methods This was a multicentre, open-label, randomised trial done at nine medical centres in Taiwan. Adult patients (>20 years) with documented *H pylori* infection were randomly assigned (1:1:1, with block sizes of six) to receive T14, C10, or BQ10. We assessed long-term outcomes (reinfection frequency, changes in the gut microbiota, antibiotic resistance, and metabolic parameters) in patients with available data, excluding all protocol violators and those with unknown post-treatment *H pylori* status. Faecal samples were collected before treatment and 2 weeks, 2 months, and at least 1 year after eradication therapy. Amplification of the V3 and V4 hypervariable regions of the 16S rRNA was done followed by high-throughput sequencing. Susceptibility testing for faecal *Escherichia coli* and *Klebsiella pneumoniae* was done. This trial is complete and registered with ClinicalTrials.gov, NCT01906879.

Findings Between July 17, 2013, and April 20, 2016, 1620 participants were randomly assigned to the three treatment groups (540 [33%] per group). 1214 (75%) attended 1-year follow-up and are included in this analysis. Compared with baseline, alpha diversity was significantly reduced 2 weeks after T14 (p=0·0002), C10 (p<0·0001), and BQ10 (p<0·0001) treatment. Beta diversity was also significantly altered 2 weeks after T14 (p=0·0010), C10 (p=0·0001), and BQ10 (p=0·0001). Alpha diversity and beta diversity were restored at week 8 (p=0·14 and p=0·918, respectively) and 1 year (p=0·14 and p=0·918) after T14, but were not fully recovered at week 8 and after 1 year in patients treated with C10 (p=0·0001 and p=0·013 at week 8; p=0·019 and p=0·064 at 1 year) and BQ10 (p<0·0001 and p=0·0002; p=0·001 and p=0·029). A transient increase at week 2 after T14 and C10 of the resistance rates of *E coli* to ampicillin-sulbactam (12% [15/127] to 66% [38/58] for T14, 7% [10/135] to 64% [28/44] for C10), cefazolin (13% [16/127] to 43% [25/58] for T14, 10% [13/135] to 41% [18/44] for C10), cefmetazole (8% [10/127] to 26% [15/58] for T14, 4% [5/135] to 18% [8/44] for C10), levofloxacin (8% [10/127] to 35% [20/58] for T14, 7% [10/135] to 32% [14/44] for C10), gentamicin (13% [19/146] to 47% [27/58] for T14, 15% [22/149] to 45% [20/44] for C10), and trimethoprim–sulfamethoxazole (33% [48/146] to 86% [50/58] for T14, 28% [42/148] to 86% [38/44] for C10; p<0·05 in paired samples in the above analyses) returned to basal state at week 8 and after 1 year. Although bodyweight and body-mass index slightly increased, there were significant improvements in metabolic parameters, with a decrease in insulin resistance, triglycerides, and LDL and an increase in HDL. Overall, there was no significant change in the prevalence of metabolic syndrome at week 8 and 1 year after T14, C10, and BQ10.

Interpretation Eradication of *H pylori* infection has minimal disruption of the microbiota, no effect on antibiotic resistance of *E coli*, and some positive effects on metabolic parameters. Collectively, these results lend support to the long-term safety of *H pylori* eradication therapy.

Funding National Taiwan University Hospital and Ministry of Science and Technology of Taiwan.

Introduction

Eradication of *Helicobacter pylori* infection reduces the recurrence of peptic ulcer disease and the incidence of gastric cancer.[1–4] Triple therapy comprising a proton pump inhibitor (PPI), amoxicillin and clarithromycin, concomitant therapy (PPI plus amoxicillin, clarithro-

Lancet Infect Dis 2019; 19: 1109–20

See **Comment** page 1042

*Contributed equally

†Co-senior authors

Division of Gastroenterology and Hepatology, Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan (J-M Liou MD, Chieh-C Chen MD, M-J Chen, Chien-C Chen MD, W-F Hsu MD, Y-N Chen MD, M-S Wu MD); **Department of Internal Medicine, National Taiwan University College of Medicine, Taipei, Taiwan** (J-M Liou, Chieh-C Chen, M-J Chen, Chien-C Chen, W-F Hsu, Y-N Chen, M-S Wu, Y-J Fang MD, J-Y Lee MD, T-H Yang MD); **Department of Internal Medicine, National Taiwan University Cancer Center, Taipei, Taiwan** (J-M Liou, W-F Hsu, Y-N Chen); **Bioinformatics and Biostatistics Core, Centers of Genomic and Precision Medicine** (C-M Chang PhD, T-P Lu PhD, E Y Chuang ScD), **Institute of Epidemiology and Preventive Medicine, Department of Public Health** (T-P Lu), **and Graduate Institute of Biomedical Electronics and Bioinformatics** (E Y Chuang), **National Taiwan University, Taipei, Taiwan; Department of Internal Medicine, National Taiwan University Hospital, Yun-Lin Branch, Yun-Lin, Taiwan** (Y-J Fang, J-Y Lee, T-H Yang); **Division of Gastroenterology, Department of Internal Medicine, Taitung Mackay Memorial Hospital, Taitung, Taiwan** (M-J Bair MD); **Mackay Medical College, New Taipei City, Taiwan** (M-J Bair); **Division of Gastroenterology and Hepatology, Department of Internal medicine, Chia-Yi Christian Hospital, Chia-Yi, Taiwan** (P-Y Chen MD,

C-Y Chen MD); **School of Medicine, College of Medicine,** (C-Y Chang MD, Y-C Hsu MD), **Department of Internal Medicine** (C-Y Chang, Y-C Hsu), **Fu Jen Catholic University Hospital, New Taipei City, Taiwan; Department of Medicine, National Yang-Ming University, School of Medicine and Taipei Veterans General Hospital, Taipei, Taiwan** (J-C Luo MD); **Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan** (J-Y Wu MD); **Digestive Medicine Center, China Medical University Hospital, Taichung, Taiwan** (J-T Lin MD); **and Microbiome Research Centre, St George & Sutherland Clinical School, University of New South Wales, Sydney, NSW, Australia** (E M El-Omar MD)

Correspondence to: Prof Ming-Shiang Wu, Department of Internal Medicine and Primary Care Medicine, National Taiwan University Hospital, National Taiwan University, College of Medicine, Taipei 10002, Taiwan
mingshiang@ntu.edu.tw

Research in context

Systematic review

We searched PubMed using the terms "*H. pylori*", "eradication", "long-term", and "microbiota", for papers published from Jan 1, 2000, to Nov 10, 2018, without language restrictions. We found no randomised trials that compared the long-term (≥1 year) changes of gut microbiota after triple therapy, concomitant therapy, and bismuth quadruple therapy for *Helicobacter pylori* eradication. We identified three studies that reported the long-term changes in the gut microbiota after *H pylori* eradication. Jakobsson and colleagues showed that the diversity of the microbiota recovered to resemble the pretreatment states, with some notable changes at the genus levels at 1 year and 4 year after triple therapy, but formal statistical analysis was not done owing to small sample size (six patients). Yap and colleagues also showed no significant differences in richness and evenness of bacterial species, but they found some notable changes at the phylum and genus levels after triple therapy (17 patients). Hsu and colleagues reported that the relative abundances of all phyla restored to the amounts at baseline at 48 weeks after bismuth quadruple therapy (11 patients). We identified another four studies that only reported the short-term changes in the gut microbiota after *H pylori* eradication (20–70 patients each). These studies showed significant changes in diversity shortly after *H pylori* eradication.

We did a further PubMed search using the terms "*H. pylori*", "eradication", "long-term", and "resistance", for papers published up to Nov 10, 2018, without language restrictions. We identified one brief communication that compared clarithromycin resistance of enterococci in five patients before and 1 year after triple therapy with five controls who did not receive antibiotics. In that study, numerically higher clarithromycin resistance prevalence was observed in patients who received triple therapy (two [50%] of four), compared with none of the controls. We searched the Web of Science to identify articles that cited this study and identified another study that compared clarithromycin resistance rates of *Staphylococcus* spp, *Streptococcus* spp, *Enterococcus* spp, and *Bacteroides* spp in 85 patients who received triple therapy and 12 controls who did not. They reported numerically higher resistance rates of staphylococcus against clarithromycin in patients who received triple therapy at 1 year (20 [34%] of 58), compared with the control group (two [20%] of ten). However, the difference was not significant.

Finally, we searched PubMed using the terms "*H. pylori*", "eradication", and "insulin resistance" for papers published up to Nov 10, 2018, without language restrictions. We identified six prospective and retrospective studies that reported the short-term changes (6–12 weeks) of insulin resistance after *H pylori* eradication. These studies showed contradictory results. None of them assessed the long-term changes of insulin resistance after *H pylori* eradication.

Added value of this trial

To our knowledge, this is the first large-scale randomised trial to show the distinct short-term and long-term effects of triple therapy, concomitant therapy, and bismuth quadruple therapy on the gut microbiota, antibiotic resistance, and metabolic parameters. We found that short-term perturbation of gut microbiota and short-term increase of antibiotic resistance of *E coli* were restored at 1 year after *H pylori* eradication. However, the speed and extent of restoration of gut microbiota varied with regimens. Whereas the gut microbiota was less perturbed by triple therapy, bismuth quadruple therapy induced minimal increase in the antibiotic resistance of *E coli*. Although there were trivial increases in body-mass index and bodyweight, insulin resistance and triglyceride concentrations decreased, indicating potential beneficial metabolic effects after *H pylori* eradication.

Interpretation of all the available evidence

Eradication of *H pylori* infection has minimal disruption of the microbiota, no effect on antibiotic resistance of *E coli*, and some positive effects on metabolic parameters. These findings collectively lend support to the long-term safety of *H pylori* eradication therapy. Taken together with our previous study, bismuth quadruple therapy is recommended in regions with clarithromycin resistance of greater than 15% because it is more effective than triple therapy, its efficacy is less affected by clarithromycin resistance, and it exerts minimal effects on the antibiotic resistance of *E coli* and *Klebsiella pneumoniae*. Triple therapy for 14 days exerts minimal perturbation of gut microbiota and might be an alternative therapy in regions with clarithromycin resistance of less than 10–15%.

mycin, and metronidazole), and bismuth quadruple therapy (PPI plus bismuth, tetracycline, and metronidazole) are the most commonly used regimens for *H pylori* eradication.[1,5–7] However, there are several concerns and barriers regarding the widespread use of antibiotics as a preventive measure against gastric cancer, including the emergence of antibiotic resistance,[8–12] the perturbation of gut microbiota,[13–16] and the development of metabolic syndrome after *H pylori* eradication.[17–20] Yet, few studies have addressed the long-term effect of *H pylori* eradication on these important issues. Emerging evidence shows that dysbiosis of the gut microbiota is associated with metabolic syndrome, diabetes, and cardiovascular diseases.[21–23] The association of *H pylori* and these same extragastric diseases might also be attributed to the gut microbiota.[17–20] Antibiotics are important modulators of the gut microbiome,[24] but different antibiotics might exert differential effects on the gut microbiota and antibiotic resistance.[24,25] Some small-scale studies showed that short courses of triple therapy might lead to long-term dysbiosis of gut microbiota.[13–16] However, the short-term and long-term

effects of different eradication regimens on the gut microbiota, antibiotic resistance, and metabolic parameters have not been reported. In a multicentre, randomised trial in Taiwan,[7] we showed that 10-day bismuth quadruple therapy (90·4% [488/540], 95% CI 87·6–92·6), but not 10-day concomitant therapy (85·9% [464/540], 82·7–88·6), was superior to 14-day triple therapy (83·7% [452/540], 80·4–86·6) in the first-line treatment of *H pylori* infection. The prespecified secondary endpoints of this trial, including the long-term effect of the three regimens on the gut microbiota, metabolic parameters, and antibiotic resistance of gut microbiota, are presented in this Article.

Methods

Study design and patients

Detailed methods for this multicentre, open-label, randomised trial were published in our previous report for the primary outcome and short-term secondary outcomes.[7] In brief, adult patients (>20 years) with at least two positive tests among histology, culture, rapid urease test, and serology were eligible (criterion 1). Asymptomatic patients with a single positive ^{13}C-urea breath test (^{13}C-UBT) were also eligible (criterion 2). Patients with any one of the following criteria were excluded from the study: history of gastrectomy, previous eradication therapy for *H pylori*, contraindication or previous allergic reactions to the study drugs, severe concurrent diseases or malignancy, pregnant or lactating women, and patients who could not give informed consent. Written informed consent was obtained from all patients before enrolment, and the trial was approved by the Institutional Review Board of each participating hospital.

Randomisation and masking

Eligible patients were randomly assigned to receive one of three regimens: triple therapy for 14 days (T14; amoxicillin 1 g, clarithromycin 500 mg, and lansoprazole 30 mg for 14 days [all given twice daily]), concomitant therapy for 10 days (C10; amoxicillin 1 g, clarithromycin 500 mg, metronidazole 500 mg, and lansoprazole 30 mg for 10 days [all given twice daily]), or bismuth quadruple therapy for 10 days (BQ10; bismuth tripotassium dicitrate 300 mg [KCB FC Tablets, Swiss Pharm, Taiwan] four times a day, tetracycline 500 mg four times a day, metronidazole 500 mg three times a day, and lansoprazole 30 mg twice daily, for 10 days). A computer-generated block randomisation sequence with a block size of six was concealed in an opaque envelope. An independent research assistant (Yu-Chung Huang; National Taiwan University Hospital, Taiwan) kept the envelope until intervention was assigned. All investigators were blind to the randomisation sequence.

Procedures

^{13}C-UBT was used to establish the *H pylori* status at least 6 weeks and 1 year after completion of treatment. All patients were asked to stop PPI and histamine-2 blockers for at least 2 weeks before ^{13}C-UBT. Stool specimens for faecal microbiota analysis and culture and susceptibility testing were collected at baseline (before treatment), at week 2, week 8, and 1 year after eradication therapy. The stool samples were collected into a DNA stabiliser kit (Stratec Biomedical; Birkenfeld, Germany) for microbiota analysis and another swab culture tube for culture and susceptibility testing. The liquid DNA stabilisation buffers preserve the microorganism titre and prelyses bacteria through the inactivation of DNases and prevent degradation of DNA. Participants were asked to return the faecal specimen to the research assistant in the hospital on the day of specimen collection. The stabilised samples were then stored in a refrigerator at –80°C immediately. The QIAamp Fast DNA Stool Mini Kit (Qiagene, MD, USA) was used to extract the genomic DNA of faecal microbiota. High throughput sequencing of 16S rRNA was done by means of the Illumina miseq. Detailed methods are shown in the appendix (p 3).

See Online for appendix

Stool culture and susceptibility testing of *Escherichia coli* and *Klebsiella pneumoniae* were done at the Union Clinical Laboratory (Taipei, Taiwan), which has been accredited by the College of American Pathologists. The stool samples were submitted to a blood agar–eosin-methylene blue agar mixed plate at 35°C according to the standard procedures. The *E coli* and *K pneumoniae* colonies identified by matrix-assisted laser desorption ionisation Biotyper system (Bruker, Germany) were submitted for subculture and susceptibility testing. The susceptibilities of *E coli* and *K pneumoniae* to various drugs, including ampicillin, ampicillin–sulbactam, piperacillin–tazobactam, cefazolin, cefmetazone, ceftriaxone, cefepime, ceftazidim, cefepime, ciprofloxacin, levofloxacin, trimethoprim–sulfamethoxazole, gentamicin, amikacin, aztreonam, imipenem, meropenem, and ertapenem were established by means of the BD Phoenix 100 ID and AST System according to the Clinical Laboratory Standard Institute.

Bodyweight, height, waist and hip circumference, and blood pressure were measured at baseline, week 8, and 1 year after eradication therapy. Fasting glucose, total cholesterol, HDL, LDL, triglycerides, insulin, and glycated haemoglobin (HbA_{1c}) were measured at baseline, week 8, and 1 year after eradication therapy. Metabolic syndrome was defined according to the revised the National Cholesterol Education Program Adult Treatment Panel III classification,[26] with at least three of the following criteria required: abdominal obesity (waist circumference: men ≥90 cm, women ≥80 cm), high triglyceride concentrations (≥150 mg/dL), high HDL cholesterol (men ≤40 mg/dL, women ≤50 mg/dL), high systolic blood pressure (≥135/85 mm Hg or taking anti-hypertensive drugs), and high fasting glucose (≥100 mg/dL). Insulin resistance was calculated according to the homeostasis model assessment, with HOMA-IR defined as fasting glucose (in mg/dL) × fasting insulin (mIU/L)/405.

	T14	C10	BQ10
Baseline characteristics in patients receiving urea breath test at 1 year			
Sex			
Female	213/408 (52%)	211/410 (51%)	207/396 (52%)
Male	195/408 (48%)	199/410 (49%)	189/396 (48%)
Age, years	53·4 (12·6)	52·8 (11·4)	53·1 (11·5)
Duodenal ulcer	89/408 (22%)	82/410 (20%)	89/396 (22%)
Gastric ulcer	153/408 (38%)	164/410 (40%)	156/396 (39%)
Erosive oesophagitis	178/399 (45%)	176/400 (44%)	170/380 (45%)
Smoking	39/408 (10%)	42/410 (10%)	41/396 (10%)
Alcohol	34/408 (8%)	30/410 (7%)	33/396 (8%)
Oral hypoglycaemic agent use	30/403 (7%)	39/401 (10%)	42/388 (11%)
Eradication rate, ITT analysis	355/408 (87%)	371/410 (90%)	378/396 (95%)
Median follow-up, years (range)	1·2 (1·1–1·8)	1·2 (1·1–1·9)	1·2 (1·1–2·0)
Reinfection or recrudescence	12/404 (3%)	10/399 (3%)	13/392 (3%)
Annual reinfection or recrudescence prevalence	1·8%	1·6%	2·0%
Metabolic parameters			
Metabolic syndrome			
Before treatment	116/400 (29%)	129/405 (32%)	142/395 (36%)
8 weeks	89/363 (25%)	87/346 (25%)	96/342 (28%)
1 year	88/334 (26%)	88/309 (28%)	92/306 (30%)
Bodyweight, kg			
Before treatment	63·9 (12·6)	64·5 (11·7)	65·2 (12·7)
8 weeks	64·2 (12·7)	64·5 (11·9)	65·2 (12·7)
1 year	64·2 (12·7)	65·3 (11·9)	65·1 (12·4)
Body-mass index			
Before treatment	24·2 (3·7)	24·5 (3·7)	24·5 (3·9)
8 weeks	24·3 (3·8)	24·3 (3·5)	24·5 (4·0)
1 year	24·3 (3·8)	24·8 (3·8)	24·6 (3·8)
Waist circumference, cm			
Before treatment	82·1 (10·7)	82·6 (10·4)	82·6 (10·5)
8 weeks	82·9 (10·8)	82·5 (10·4)	82·6 (10·4)
1 year	84·0 (10·9)	84·0 (10·7)	84·2 (10·7)
Systolic blood pressure, mm Hg			
Before treatment	125·7 (16·6)	125·9 (17·9)	127·2 (17·2)
8 weeks	124·2 (17·0)	125·3 (17·3)	124·6 (18·1)
1 year	126·0 (16·9)	126·3 (17·4)	126·0 (16·6)
Diastolic blood pressure, mm Hg			
Before treatment	77·1 (11·6)	76·5 (11·6)	77·2 (11·9)
8 weeks	76·0 (12·2)	75·6 (12·1)	75·9 (13·1)
1 year	76·0 (11·3)	75·4 (10·8)	75·7 (11·1)
Fasting glucose, mg/dL			
Before treatment	98·6 (36·0)	98·6 (36·0)	97·1 (28·0)
8 weeks	95·0 (19·0)	95·0 (21·0)	96·1 (24·4)
1 year	93·1 (21·2)	95·5 (23·2)	96·9 (28·2)
HbA_{1c}			
Before treatment	5·8% (0·9)	5·9% (1·0)	5·8% (0·7)
8 weeks	5·8% (0·7)	5·8% (0·8)	5·8% (0·8)
1 year	5·9% (0·8)	6·0% (0·9)	5·9% (0·9)
Total cholesterol, mg/dL			
Before treatment	194·8 (38·4)	194·0 (35·0)	195·0 (37·0)
8 weeks	197·7 (38·0)	198·6 (36·0)	198·7 (36·7)
1 year	197·0 (36·6)	196·7 (37·5)	196·0 (38·9)

(Table 1 continues on next page)

Outcomes

The long-term outcomes assessed in this analysis were changes in faecal microbiota, antibiotic resistance of *E coli* and *K pneumoniae* in faeces, and metabolic parameters before and after *H pylori* eradication therapy.

Statistical analysis

In this per-protocol analysis, we excluded all protocol violators (ie, patients not taking at least 80% of treatment drugs) or those with unknown post-treatment *H pylori* status from the original intention-to-treat population. Patients with missing data for the outcome considered were not included in the analysis for that outcome. We used the χ^2 test or Fisher's exact test for analysis of categorical data and Student's *t* test or the ANOVA test for analysis of continuous data. We used the McNemar test for analysis of changes in antibiotic resistance before and after eradication therapy by means of paired samples. 95% CIs were calculated for the reinfection prevalence of each regimen. We used SPSS (version 21) for Windows for the statistical analyses.

In the bioinformatics analysis, for each pair-end fastq, we first trimmed each sequence to 250 bp from the 5′ end and used Paired-End reAd mergeR (PEAR, version 0.9.6) to merge the pair-end sequences on the basis of approximately 40 bp of overlap between the two pair-end sequences. The merged fastq file (spanning the entire 16S rRNA gene V3–V4 region) was quality controlled by QIIME version 1.9.1. The sequences of the V3–V4 hypervariable regions were compared with the database and those with greater than 97% similarity were classified as the same operational taxonomic units (OTUs). The OTUs of each specimen were generated from non-chimeric reads by means of the UPARSE software after quality-filter. The sequences of the OTUs were compared with the Greengenes database version 13_8 by means of the UCLUST algorithm to identify the best fit global alignment taxonomy. Alpha diversity represents the richness (ie, how many different species) and evenness (ie, similar abundance or some species dominate others) of species in a microbial ecosystem. Beta diversity represents the differences in the microbial composition in one environment compared with another. The diversity indices, including the Shannon, Chaol, PD_whole tree, and observed_OTUs were calculated by means of the QIIME version 1.9.1 software. Principal coordinate analysis was used to visualise clustering of samples on the basis of their similarity matrices. The PEARANOVA on the weighted UniFrac distance matrices (a distance metric used for comparing the biological communities that accounts for the abundance of observed organisms) was computed by means of unrestricted permutation of raw data with 9999 random permutations and used to analyse the dissimilarity of microbiota between two groups. The Kruskal-Wallis and Wilcoxon tests were used to analyse the ecological similarity between and within groups.

This trial is registered with Clinical Trial.gov, NCT01906879.

Role of the funding source

The funding source had no role in study design, data collection, analysis or interpretation, report writing, or the decision to submit this paper for publication. All authors had full access to the data and participated in the decision to submit for publication.

Results

Between July 17, 2013, and April 20, 2016, we screened 5454 patients, of whom 1620 (30%) were randomly assigned to treatment (540 [33%] per group). 1214 patients had data on long-term outcomes (table 1, appendix p 4). Of 1195 successfully treated patients, reinfection or recrudescence was observed in 12 (3%) of 404 patients treated with triple therapy, ten (3%) of 399 treated with concomitant therapy, and 13 (3%) of 392 treated with quadruple therapy after 1·6 years (p=0·80; table 1).

At baseline, there were no significant differences in alpha diversity between the three treatment groups (p=0·50; figure 1A; appendix p 5). However, at week 2, the alpha diversity was lower in patients treated with concomitant therapy ($p=7·5\times10^{-11}$) and bismuth quadruple therapy ($p=2·3\times10^{-7}$) compared with those treated with triple therapy (figure 1B; appendix p 5). Although there was a trend for restoration of microbiota with time, the alpha diversity was still significantly lower in patients treated with concomitant therapy (p=0·0081) and quadruple therapy (p=0·0004), compared with those treated with triple therapy at week 8 (figure 1C, appendix p 5). The alpha diversity was still significantly lower in patients treated with concomitant therapy (p=0·013) and bismuth quadruple therapy (p=0·014), compared with those treated with triple therapy at 1 year (figure 1D; appendix p 5).

There were no significant differences in the beta diversity between the three treatment groups before treatment (figure 1E). However, there were significant differences in the beta diversity in patients treated with triple therapy, concomitant therapy, and quadruple therapy at week 2 (figure 1F), week 8 (figure 1G), and 1 year (figure 1H).

Compared with baseline, the species richness (alpha diversity) was significantly reduced at 2 weeks (p=0·0002), but the richness returned to baseline at week 8 (p=0·14) and 1 year (p=0·81) after triple therapy (figure 2A; appendix p 6). Compared with baseline, there were significant differences in the faecal microbiota structure (beta diversity) at 2 weeks (p=0·0010). However, beta diversity was restored at week 8 (p=0·92) and after 1 year (p=0·44; figure 2B).

Compared with baseline, alpha diversity was significantly reduced at week 2 ($p=5·1\times10^{-15}$), week 8 (p=0·0001), and 1 year (p=0·019) after concomitant treatment (figure 2C; appendix p 11). There were significant differences in the beta diversity at week 2 (p=0·0001) and week 8 (p=0·013) compared with baseline. However, beta diversity appeared to be restored after 1 year (p=0·064; figure 2D).

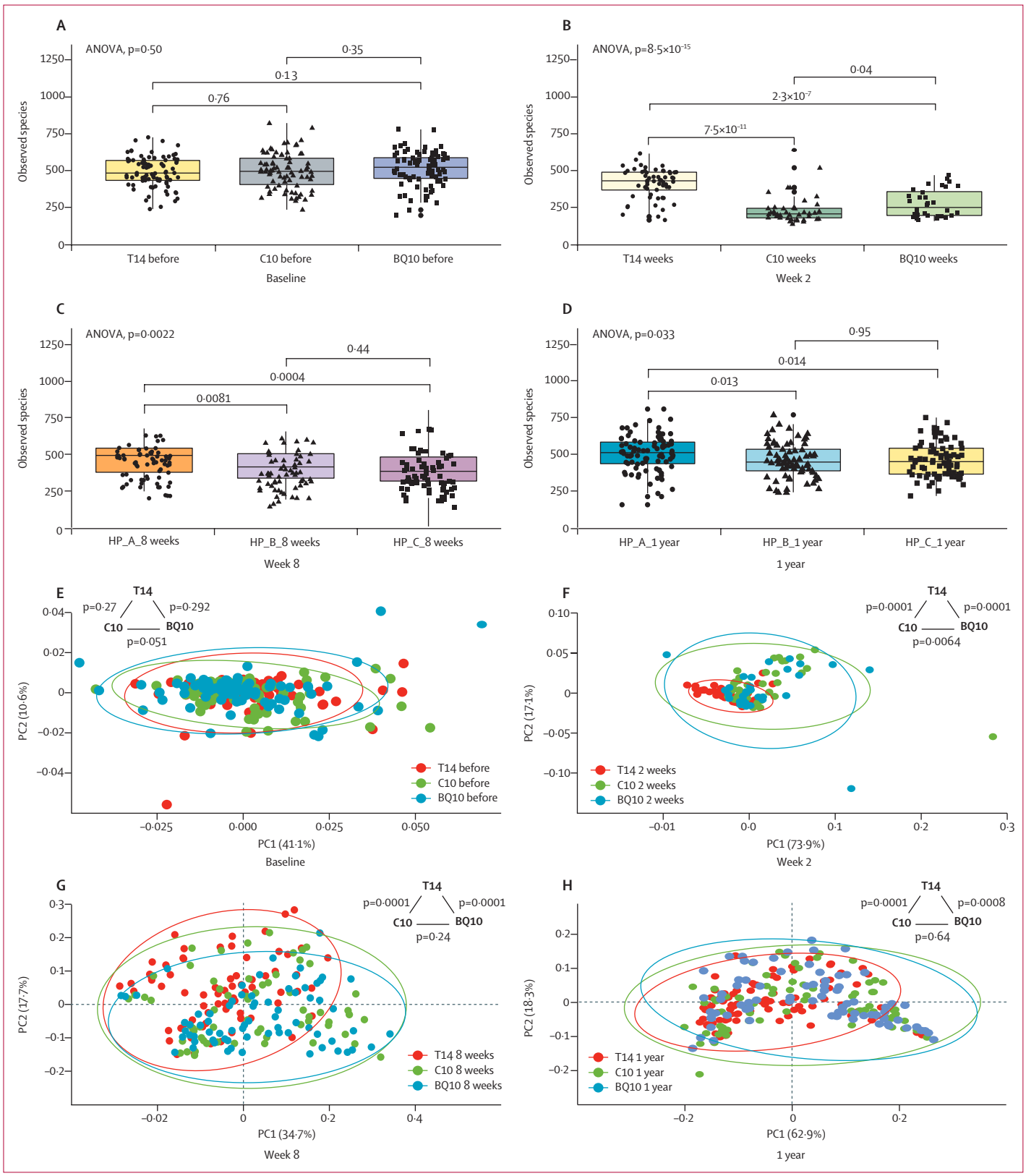
Compared with baseline, the alpha diversity was significantly reduced at 2 weeks ($p=7·6\times10^{-13}$), 2 months ($p=2·1\times10^{-8}$), and 1 year (p=0·0010) after quadruple therapy (figure 2E; appendix p 11). Compared with baseline, there were significant differences in the beta diversity at week 2 (p=0·0001), week 8 (p=0·0002), and 1 year (p=0·029) after quadruple therapy (figure 2F).

There was significant reduction in the abundance of fusobacteria at week 2 compared with baseline in patients treated with triple therapy (appendix p 29). However, the changes returned to basal state at week 8 and 1 year. There were no significant differences in the relative abundance of Bacteroidetes, Proteobacteria, and Firmicutes, and at week 2, week 8, and 1 year, compared with baseline at phylum level in patients treated with triple therapy (appendix p 29). In patients treated with concomitant therapy and quadruple therapy, there was a significant decrease in the relative abundance of Bacteroidetes, Firmicutes, and fusobacteria, whereas there was a significant increase in the relative abundance of Proteobacteria at week 2. However, these changes were restored at week 8 and after 1 year (appendix p 29).

	T14	C10	BQ10
(Continued from previous page)			
Triglyceride, mg/dL			
Before treatment	128·1 (94·8)	139·1 (106·0)	141·2 (97·6)
8 weeks	123·3 (89·8)	123·4 (81·4)	127·1 (75·4)
1 year	119·0 (75·8)	115·4 (63·0)	127·0 (81·6)
Low density lipoprotein, mg/dL			
Before treatment	122·2 (33·3)	121·0 (31·0)	122·3 (33·9)
8 weeks	123·6 (33·8)	124·7 (33·4)	130·2 (72·6)
1 year	123·9 (34·0)	124·1 (33·5)	123·9 (34·3)
High density lipoprotein, mg/dL			
Before treatment	54·2 (16·3)	53·0 (16)	52·1 (16·1)
8 weeks	56·7 (16·4)	56·5 (16·3)	54·3 (15·4)
1 year	58·2 (18·8)	55·8 (17·5)	52·7 (16·3)
Insulin, mIU/mL			
Before treatment	13·4 (17·7)	13·6 (7·7)	15·9 (23·7)
8 weeks	9·7 (8·5)	10·7 (9·5)	10·2 (7·4)
1 year	10·3 (8·1)	10·3 (10·4)	10·3 (8·9)
HOMA-IR, mIU/mL × mmol/L			
Before treatment	3·6 (6·9)	3·7 (6·7)	4·3 (8·5)
8 weeks	2·4 (2·7)	2·6 (2·9)	2·5 (2·2)
1 year	2·4 (2·2)	2·5 (3·2)	2·6 (2·8)

Data are n/N (%) for proportional data and mean (SD) for continuous data, unless specified otherwise. T14=triple therapy for 14 days. C10=concomitant therapy for 10 days. BQ10=bismuth quadruple therapy. HbA_{1c}=glycated haemoglobin. HOMA-IR=homoeostatic model assessment for insulin resistance. ITT=intention to treat.

Table 1: **Demographic characteristics of patients with long-term outcomes and changes in metabolic parameters**


A
ANOVA, p=0·50
0·35
0·13
0·76
Observed species
T14 before
C10 before
BQ10 before
Baseline
B
ANOVA, p=8·5×10⁻¹⁵
0·04
2·3×10⁻⁷
7·5×10⁻¹¹
T14 weeks
C10 weeks
BQ10 weeks
Week 2
C
ANOVA, p=0·0022
0·44
0·0004
0·0081
HP_A_8 weeks
HP_B_8 weeks
HP_C_8 weeks
Week 8
D
ANOVA, p=0·033
0·95
0·014
0·013
HP_A_1 year
HP_B_1 year
HP_C_1 year
1 year
E
T14
p=0·27
p=0·292
C10
BQ10
p=0·051
PC2 (10·6%)
PC1 (41·1%)
T14 before
C10 before
BQ10 before
Baseline
F
T14
p=0·0001
p=0·0001
C10
BQ10
p=0·0064
PC2 (17·1%)
PC1 (73·9%)
T14 2 weeks
C10 2 weeks
BQ10 2 weeks
Week 2
G
T14
p=0·0001
p=0·0001
C10
BQ10
p=0·24
PC2 (17·7%)
PC1 (34·7%)
T14 8 weeks
C10 8 weeks
BQ10 8 weeks
Week 8
H
T14
p=0·0001
p=0·0008
C10
BQ10
p=0·64
PC2 (18·3%)
PC1 (62·9%)
T14 1 year
C10 1 year
BQ10 1 year
1 year

Significant perturbations of microbiota but distinct patterns of perturbation were observed at genus level at week 2 for the three regimens (appendix p 32). Most of the changes in the relative abundance at genus level were restored but some of the changes were not yet fully recovered at 8 weeks (appendix p 32) and even after 1 year (appendix p 32).

Before treatment, there were no significant differences in the prevalence of resistance to the antibiotics used in the three regimens (table 2; appendix pp 13, 16). At week 2, the prevalence of antibiotic resistance of *E coli* to ampicillin (p<0·0001), ampicillin–sulbactam (p=0·0004), piperacillin–tazobactam (p=0·039), cefazolin (p=0·0008), cefmetazole (p=0·049), ciprofloxacin (p=0·0016), levofloxacin (p=0·016), gentamicin (p=0·0002), and trimethoprim–sulfamethoxazole (p=0·0070) were significantly higher in patients treated with triple therapy and concomitant therapy, compared with quadruple therapy. However, there were no significant differences in the prevalence of antibiotic resistance among the three treatment groups at week 8 and 1 year later, except for a borderline higher ertapenem resistance in patients treated with triple therapy, compared with concomitant and quadruple therapies at week 8 (p=0·046). The changes of antibiotic resistance of *K pneumoniae* were similar to those of *E coli* (table 2; appendix p 16).

The susceptibility testing of paired *E coli* isolated from the same patient before and after each regimen were analysed by means of the McNemar test (appendix p 21).

Compared with baseline, the prevalence of antibiotic resistance significantly increased for ampicillin (p<0·0001), ampicillin–sulbactam (p<0·0001), cefazolin (p=0·0024), cefmetazole (p<0·0001), ciprofloxacin (p<0·0001), levofloxacin (p=0·0042), gentamicin (p<0·0001), and trimethoprim–sulfamethoxazole (p<0·0001) in patients treated with triple therapy at week 2 (appendix p 21). However, there were no significant differences in the prevalence of antibiotic resistance for these antibiotics at week 8 and after 1 year, compared with baseline (appendix p 21). There were no significant changes in the prevalence of antibiotic resistance for aztreonam, ceftazidime, ceftriaxone, cefotaxime, ertapenem, cefepime, imipenem, meropenem, and piperacillin–tazobactam at week 2, week 8, and after 1 year, compared with baseline.

Compared with baseline, the prevalence of antibiotic resistance significantly increased for ampicillin (p<0·0001), ampicillin–sulbactam (p<0·0001), cefazolin (p=0·013), cefmetazole (p=0·031), and trimethoprim–sulfamethoxazole (p<0·0001) in patients treated with C10 at week 2 (appendix p 21). There were non-significant increases in resistance frequency for gentamicin (p=0·057), ciprofloxacin (p=0·065), and levofloxacin (p=0·065) at week 2, compared with baseline. However, there were no significant differences in the prevalence of antibiotic resistance for these antibiotics at week 8 and after 1 year. There were no significant changes in the prevalence of antibiotic resistance for other antibiotics at week 2, week 8, and after 1 year, compared with baseline.

Compared with baseline, the prevalence of antibiotic resistance significantly increased for trimethoprim–sulfamethoxazole (p=0·0015) in patients treated with quadruple therapy at week 2, but the resistance prevalence returned to basal state at week 8 and after 1 year (appendix p 21). There were no significant changes in the prevalence of antibiotic resistance for the other antibiotics at week 2, week 8, and 1 year, compared with baseline.

There were no significant differences in the prevalence of metabolic syndrome, bodyweight, body-mass index, waist circumference, systolic or diastolic blood pressure, fasting glucose, HbA_{1C}, total cholesterol, triglyceride, LDL, HDL, insulin and HOMA-IR in patients treated with different regimens before treatment, at week 8, and after 1 year (table 1).

The changes in metabolic parameters before and after eradication within each regimen in paired samples are shown in the appendix (appendix pp 26–27). We observed a slight decrease in body-mass index and bodyweight at week 8 in patients treated with triple (mean difference 0·1 kg/m² [p=0·015] for body-mass index and 0·17 kg [p=0·047] for bodyweight), concomitant (0·15 kg/m² [p=0·0015] and 0·24 kg [p=0·012]), and quadruple (0·18 kg/m² [p=0·0027] and 0·44 kg [p=0·0011]) therapies, compared with baseline (appendix p 26). However, we observed a slight increase in the body-mass index and bodyweight at 1 year in patients treated with triple (0·2 kg/m² [p=0·0064] and 0·49 kg [p=0·0051]) and concomitant (0·21 kg/m² [p=0·0035] and 0·38 kg [p=0·025]) therapies, compared with baseline (appendix p 27). The insulin concentrations and insulin resistance were significantly reduced at week 8 in patients treated with triple (3·7 μIU/mL [p=0·0002] for insulin concentrations and 1·3 μIU/mL×mmol/L [p=0·0014] for insulin resistance), concomitant (3·8 μIU/mL [p=0·0003] and 1·4 μIU/mL×mmol/L [p=0·0002]), and quadruple (5·0 μIU/mL [p=0·0001] and 1·7 μIU/mL×mmol/L [p=0·0005]) therapies, compared with baseline (appendix p 26). The insulin concentrations and insulin resistance were still significantly reduced at 1 year in patients treated with triple (4·0 μIU/mL [p=0·0003] and 1·2 μIU/mL×mmol/L [p=0·0004]), concomitant (4·0 μIU/mL [p=0·0002] and 1·5 μIU/mL×mmol/L [p=0·0001]) and quadruple (5·2 μIU/mL [p<0·0001] and 1·6 μIU/mL×mmol/L [p=0·0008]) therapies, compared

Figure 1: **Comparison of the diversity between the three regimens before and after eradication therapy**
Alpha diversity (A–D) represents the richness (ie, how many different species) and evenness (ie, similar abundance or some species dominating others) of species in a microbial ecosystem. Beta diversity (E–H; principal coordinate analysis) represents the differences in the microbial composition in one environment compared with another. T14=triple therapy for 14 days. C10=concomitant therapy for 10 days. BQ10=bismuth quadruple therapy for 10 days.

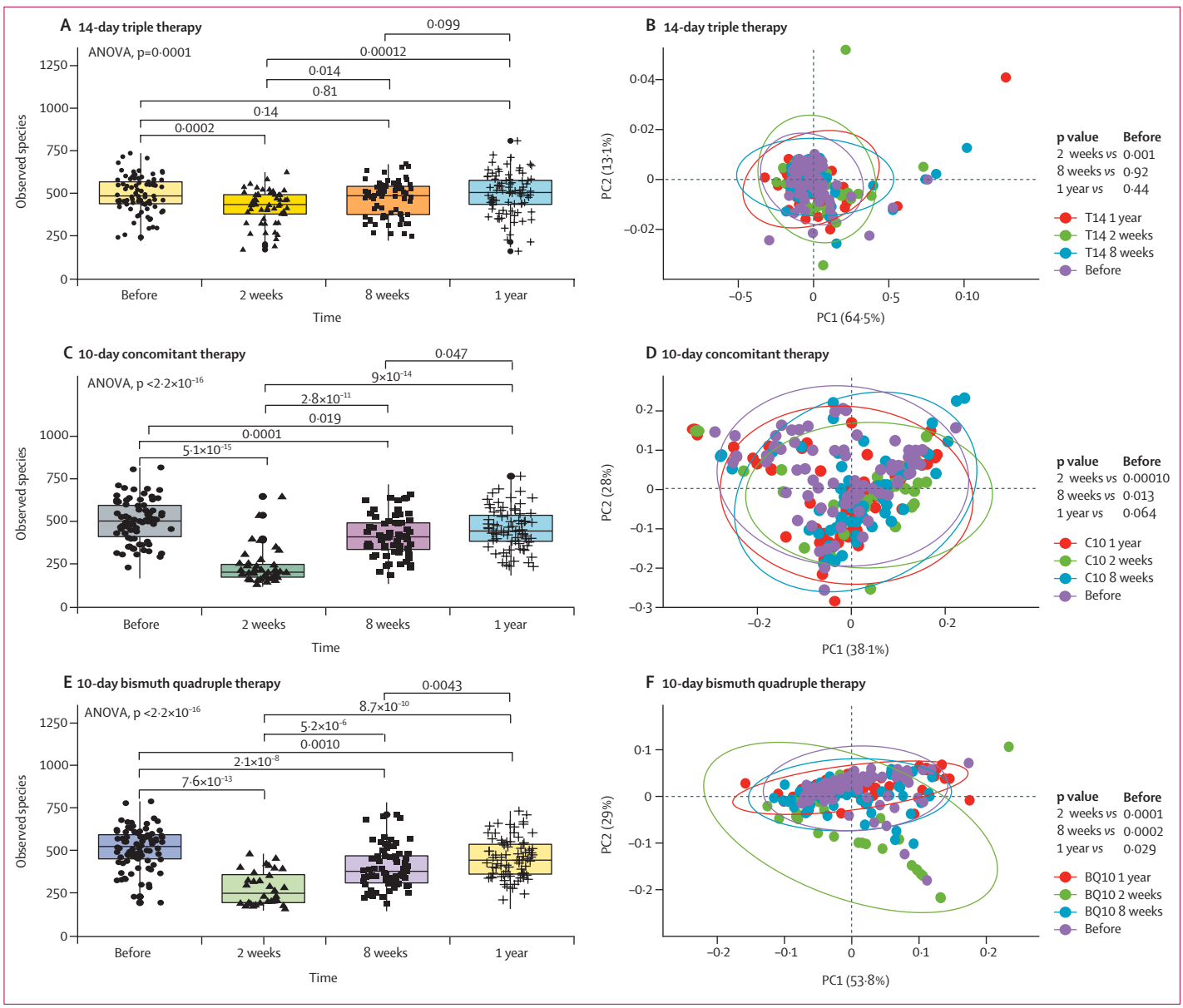


***Figure 2*: Changes in the diversity before treatment, at week 2, week 8, and 1 year within each regimen**
Alpha diversity (A, C, E) and beta diversity (B, D, F; principal coordinate analysis). T14=triple therapy for 14 days. C10=concomitant therapy for 10 days. BQ10=bismuth quadruple therapy for 10 days.

with baseline (appendix p 27). Fasting glucose concentrations were reduced at 1 year in patients treated with triple and concomitant therapies (appendix pp 26–27). However, HbA_{1c} concentrations were not significantly changed at week 8 and 1 year for the three regimens (appendix pp 26–27). There were small increases in HDL concentrations and small decreases in triglyceride concentrations at week 8 and 1 year in patients treated with the three regimens, compared with baseline (appendix pp 26–28). However, there were no significant changes in the prevalence of metabolic syndrome at week 8 and 1 year in patients treated with the three regimens, compared with baseline (appendix pp 26–27).

Discussion

To our knowledge, this is the first large-scale, randomised trial to show the distinct short-term and long-term effects of triple therapy, concomitant therapy, and bismuth quadruple therapy on the gut microbiota, antibiotic resistance, and metabolic health parameters (table 3). As expected with any antibiotic treatment, we observed significant perturbations of the gut microbiota at the end (week 2) of *H pylori* eradication therapy with the three regimens, but to a significantly greater extent for concomitant therapy and bismuth quadruple therapy than triple therapy. However, alpha and beta diversities were restored at week 8 and 1 year in patients treated

	Escherichia coli				*Klebsiella pneumoniae*			
	T14	C10	BQ10	p value	T14	C10	BQ10	p value
Ampicillin-sulbactam								
Before	12% (15/127)	7% (10/135)	7% (9/124)	0·58	4% (3/77)	11% (8/72)	6% (5/81)	0·45
2 weeks	66% (38/58)	64% (28/44)	12% (7/57)	0·0004	53% (16/30)	63% (30/48)	39% (19/49)	0·070
8 weeks	12% (22/190)	11% (21/188)	9% (17/187)	0·84	6% (5/90)	4% (4/114)	12% (12/98)	0·044
1 year	10% (19/193)	7% (14/190)	11% (18/170)	0·23	6% (5/85)	5% (4/81)	6% (5/81)	0·84
Cefazolin								
Before	13% (16/127)	10% (13/135)	9% (11/124)	0·57	3% (2/77)	3% (2/72)	3% (2/81)	0·99
2 weeks	43% (25/58)	41% (18/44)	12% (7/57)	0·0008	30% (9/30)	33% (16/48)	14% (7/49)	0·076
8 weeks	14% (27/190)	10% (18/188)	9% (17/187)	0·47	2% (2/90)	4% (5/114)	9% (9/98)	0·22
1 year	7% (13/193)	5% (10/190)	10% (17/170)	0·39	7% (6/85)	2% (2/81)	4% (3/81)	0·37
Ceftazidime								
Before	7% (10/146)	3% (4/149)	2% (3/143)	0·17	1% (1/88)	0% (0/81)	1% (1/98)	0·64
2 weeks	14% (8/58)	16% (7/44)	7% (4/57)	0·25	23% (7/30)	21% (10/48)	8% (4/49)	0·38
8 weeks	6% (12/191)	3% (6/189)	3% (6/187)	0·28	0% (0/90)	1% (1/115)	1% (1/98)	0·44
1 year	5% (9/193)	4% (7/190)	4% (7/169)	0·36	1% (1/85)	0% (0/81)	0% (0/81)	0·38
Levofloxacin								
Before	8% (10/127)	7% (10/135)	6% (7/124)	0·63	0% (0/77)	1% (1/72)	1% (1/81)	0·60
2 weeks	34% (20/58)	32% (14/44)	11% (6/57)	0·016	10% (3/30)	13% (6/48)	12% (6/49)	0·54
8 weeks	9% (18/190)	11% (20/188)	9% (16/187)	0·074	1% (1/90)	1% (1/114)	3% (3/98)	0·41
1 year	10% (20/193)	11% (20/190)	6% (11/170)	0·16	2% (2/85)	0% (0/81)	1% (1/81)	0·21
Trimethoprim–sulfamethoxazole								
Before	33% (48/146)	28% (42/148)	37% (53/143)	0·29	6% (5/87)	6% (5/81)	7% (7/98)	0·92
2 weeks	86% (50/58)	86% (38/44)	65% (37/57)	0·0070	73% (22/30)	65% (31/48)	61% (30/49)	0·54
8 weeks	41% (79/191)	39% (74/189)	33% (62/187)	0·24	7% (6/90)	13% (15/115)	18% (18/98)	0·057
1 year	31% (59/193)	30% (57/190)	35% (60/170)	0·50	4% (3/85)	11% (9/81)	7% (6/81)	0·17
Gentamicin								
Before	13% (19/146)	15% (22/149)	10% (14/143)	0·43	1% (1/88)	4% (3/81)	2% (2/98)	0·60
2 weeks	47% (27/58)	45% (20/44)	12% (7/57)	0·0002	37% (11/30)	40% (19/48)	33% (16/49)	0·87
8 weeks	17% (33/191)	11% (21/189)	13% (24/187)	0·26	3% (3/90)	3% (3/115)	6% (6/98)	0·170
1 year	9% (18/193)	7% (14/190)	9% (15/170)	0·66	2% (2/85)	4% (3/81)	0% (0/81)	0·40
Imipenem								
Before	1% (1/146)	1% (2/149)	0% (0/143)	0·38	0% (0/88)	1% (1/81)	0% (0/98)	0·32
2 weeks	0% (0/58)	2% (1/44)	0% (0/57)	0·35	0% (0/30)	0% (0/48)	0% (0/49)	NA
8 weeks	1% (1/191)	0% (0/189)	1% (1/187)	0·61	0% (0/90)	1% (1/115)	1% (1/98)	0·65
1 year	0% (0/193)	0% (0/190)	1% (1/170)	0·51	1% (1/85)	0% (0/81)	0% (0/81)	0·38

T14=triple therapy for 14 days. C10=concomitant therapy for 10 days. BQ10=bismuth quadruple therapy. NA=not available. The χ^2 test or Fisher's exact test were used to compare the antibiotic resistance prevalence in the T14, C10, and BQ10 at a given time. For example, the ampicillin-sulbactam resistance prevalences were significantly different among the three treatment groups at week 2 (p<0·0001). However, there were no significant differences in the ampicillin-sulbactam resistance prevalences among the three treatment groups at week 8 (p=0·84) and 1 year (p=0·23).

Table 2: **Prevalence of antibiotic resistance of *Escherichia coli* and *Klebsiella pneumoniae* before and after *Helicobacter pylori* eradication**

with triple therapy. Although alpha and beta diversities were not yet fully restored at 1 year in patients treated with concomitant therapy and bismuth quadruple therapy, we observed a trend of gradual restoration with time. There were transient increases in the resistance to *E coli* and *K pneumoniae* to certain antibiotics at week 2 after triple therapy and concomitant therapy, but susceptibility to these antibiotics was restored at week 8 and 1 year after eradication therapy. It is noteworthy that the antibiotic resistance rates of *E coli* were not significantly increased after bismuth quadruple therapy at week 2, week 8, and 1 year. Although there were trivial but significant increases in the body-mass index and bodyweight at 1 year, these changes are clinically irrelevant (0·2–0·5 kg). More importantly, we observed some beneficial changes, including significant reductions in insulin concentrations, insulin resistance, and triglycerides and an increase in HDL at week 8 and 1 year after eradication therapies. Overall, we show that *H pylori* eradication therapy does not increase the prevalence of metabolic syndrome in the short and long-term. The results from this trial collectively provide an important

	14-day triple therapy (T14)			10-day concomitant therapy (C10)			10-day bismuth quadruple therapy (BQ10)		
	Week 2	Week 8	1 year	Week 2	Week 8	1 year	Week 2	Week 8	1 year
Gut (faecal) microbiota									
Alpha diversity	Reduced	Restored	Restored	Reduced	Reduced	Reduced	Reduced	Reduced	Reduced
Beta diversity	Altered	Restored	Restored	Altered	Altered	Altered	Altered	Altered	Altered
Phylum	Fuso↓	Restored	Restored	Pro↑, Fuso↓, Fir↓, Bac↓	Restored	Restored	Pro↑, Fuso↓, Fir↓, Bac↓	Restored	Restored
Altered genus	47% (29/62)	29% (18/62)	29% (18/62)	85% (53/62)	24% (15/62)	23% (14/62)	77% (48/62)	45% (28/62)	29% (18/62)
Antibiotic resistant rate of *Escherichia coli*	AMP↑, SAM↑, TZP↑, CFZ↑, CMZ↑, CIP↑, LVX↑, GEN↑, TMP/SMX↑	Returned to basal state	Returned to basal state	AMP↑, SAM↑, TZP↑, CFZ↑, CMZ↑, CIP↑, LVX↑, GEN↑, TMP/SMX↑	Returned to basal state	Returned to basal state	No significant changes except TMP/SMX↑	No significant changes	No significant changes
Metabolic parameters	..	BMI↓, BW↓, insulin↓, HOMA-IR↓, HDL↑, MS↔	BMI↑, BW↑, insulin↓, glu(ac)↓, HOMA-IR↓, HDL↑, MS↔	..	BMI ↓, BW↓, insulin↓, HOMA-IR↓, HDL↑, TG↓, MS↔	BMI↑, BW↑, insulin↓, glu(ac)↓, HOMA-IR↓, HDL↑, TG↓, MS↔	..	BMI ↓, BW↓, insulin↓, HOMA-IR↓, HDL↑, TG↓, MS ↓	BMI↑, BW↔, insulin↓, glu(ac)↔, HOMA-IR↓, HDL↑, TG↓, MS↔
Annual reinfection rate*	..	..	1·8%	..	..	1·6%	..	..	2·0%

Fuso=fusobacterium. Pro=Proteobacterium. Fir=Firmicutes. Bac=Bacteroidetes. AMP=ampicillin. SAM=ampicillin–sulbactam. TZP=piperacillin–tazobactam. CFZ=cefazolin. CMZ=cefmetazole. CIP=ciprofloxacin. LVX=levofloxacin. GEN=gentamicin. TMP/SMX=trimethoprim–sulfamethoxazole. BMI=body-mass index. BW=bodyweight. HOMA-IR=homeostatic model assessment for insulin resistance. HDL=high density lipoprotein. TG=triglyceride. MS=metabolic syndrome. *Reinfection/recrudescence. Compared with baseline, alpha diversity and beta diversity were significantly reduced 2 weeks after T14, C10, and BQ10. Alpha diversity and beta diversity were restored at week 8 and 1 year in patients treated with T14 but were not fully recovered in patients treated with C10 and BQ10 at week 8 and 1 year. The abundance of fusobacterium was significantly reduced at week 2 but was restored at week 8 and 1 year in the T14 group. The abundance of Proteobacterium was increased, but the abundances of fusobacterium, Firmicutes, and Bacteroidetes were reduced in the C10 and BQ10 groups at week 2, but the changes were restored at week 8 and 1 year in both groups. There were significant alterations in the abundance at genus level in the T14, C10, and BQ10 groups at week 2, week 8 and 1 year, compared to baseline. The transient increase of the resistance rates of *E coli* to penicillin derivatives, cefazolin, cefmetazole, fluoroquinolones, gentamicin, and trimethoprim–sulfamethoxazole after T14 and C10 at week 2 returned to basal state at week 8 and 1 year. Although bodyweight and body-mass index slightly increased, there were significant improvements in metabolic parameters with a decrease in insulin resistance, triglycerides, and low-density lipoprotein, and an increase in high density lipoprotein 1 year after T14, C10, and BQ10. Overall, there was no significant change in the prevalence of metabolic syndrome at week 8 and 1 year, after T14, C10, and BQ10.

Table 3: **Summary of the main findings**

message, namely the long-term safety of *H pylori* eradication therapy in relation to gut microbiota, antibiotic resistance, and metabolic health parameters.

Some of the previous studies have shown that different antibiotics might exert distinct effects on gut microbiota. However, most of these studies addressed only the short-term changes after antibiotic treatment and few of them addressed the long-term effect.[24] Korpela and colleagues showed that use of macrolides, but not penicillin, might induce long-term alteration of microbiota in preschool children.[25] However, whether antibiotic treatment exerts long-term alteration of gut microbiota in adults remains controversial. The changes in the gut microbiota before and after *H pylori* eradication have been reported in six small-scale studies (six to 23 cases, appendix p 35).[13–16,27,28] However, the long-term effect of *H pylori* eradication on the gut microbiota was assessed in only three studies (with a total of 34 cases, appendix p 35).[13–15] These studies were underpowered to assess the long-term alterations in gut microbiota after *H pylori* eradication. Oh and colleagues showed that the relative abundance of Firmicutes decreased and that of Proteobacteria increased immediately after triple therapy.[27] Hsu and colleagues showed that the relative abundance of Proteobacteria increased, whereas that of Bacteroidetes and Actinobacteria decreased immediately after bismuth quadruple therapy.[15] We observed significant perturbations of microbiota, with distinct patterns of perturbation at genus level, at week 2 for the three regimens. Most of these changes in the relative abundance at genus level were restored but some of the changes persisted even after 1 year. We found a greater than 30% reduction of the observed species at 1 year in, patients treated with triple (five [7%] of 72), concomitant (seven [12%] of 60), and bismuth quadruple (12 [18%] of 66) therapies. The transient decreases in the abundance of *Akkermansia* at genus level at week 2 after concomitant therapy and bismuth quadruple therapy were restored at 1 year. Yet, further in-depth sequencing is needed to assess the long-term changes in the abundance of *Akkermansia muciniphila*.

An earlier study[9] showed persistent clarithromycin resistance of enterococci in three of the five patients 1 year after triple therapy, compared with none of the five controls (appendix p 36). Jacobson and colleagues showed high amounts of the macrolide resistance gene *erm* (B) 4 years after triple therapy in three patients, compared with another three control patients.[14] In another study, the prevalence of clarithromycin resistant staphylococci and streptococci remained higher 1 year after treatment with triple therapy (85 patients; appendix p 36).[10] However, we showed that the transient increase in the antibiotic resistance of *E coli* to penicillin derivatives and first and second-generation cephalosporins after triple therapy and concomitant therapy recovered to

pretreatment concentrations at week 8 and at 1 year. Our results are in agreement with a study that showed that gut bacterial microbiota and its resistome rapidly recover to basal state amounts in healthy adults treated with amoxicillin–clavulanic acid for 1 week.[29] The resilience of gut microbiota and antibiotic resistance provide important evidence on the safety issue of *H pylori* eradication.

The effect of *H pylori* eradication on metabolic parameters remains contradictory.[17–20] Our results are in agreement with a previous trial showing a trivial increase in body-mass index after *H pylori* eradication.[30] Nevertheless, we also observed some beneficial changes in the metabolic parameters, including a decrease in insulin resistance and triglyceride concentrations and a small increase in HDL concentration. Six of eight cross-sectional studies showed an association of *H pylori* infection with insulin resistance.[17] A cohort study and a randomised trial showed that *H pylori* eradication might reduce insulin resistance 2 months after therapy, but another cohort study and randomised trial showed negative results.[17,19,20] Our trial is the first study to show that insulin resistance is reduced at week 8 and 1 year after *H pylori* eradication by means of either triple, concomitant, or quadruple therapies. The effect of *H pylori* eradication on the lipid profiles remains contradictory in the literature and few of the previous studies addressed its long-term effect.[18–20] We observed an increase in the HDL concentrations at week 8 and 1 year after *H pylori* eradication using any of the three regimens and a decrease in triglyceride concentrations at week 8 and 1 year after concomitant and bismuth quadruple therapies. These changes were not different among those with successful eradication and those with persistent *H pylori* infection, indicating that the changes might be attributed to gut microbiota rather than the disappearance of *H pylori* infection alone.[21,22] Although we observed significant changes in some individual metabolic parameters, there were no significant changes in the prevalence of metabolic syndromes at 1 year after *H pylori* eradication. Whether these changes are associated with significant clinical outcomes, such as cardiovascular events, should be assessed in future studies.

The strengths of this study include the prespecified long-term outcomes in a large-scale, randomised trial, the testing of three eradication regimens in wide use globally on these outcomes, the analysis of susceptibility testing of various antibiotics for *E coli*, and the extensive analysis of metabolic parameters. The safety issues addressed in this randomised trial can be generalised to other populations.

Nevertheless, there were some limitations to this study. First, only 75% of patients underwent the 1-year follow-up. Second, gut microbiota was available for analysis in a subgroup of patients. Yet, the sample size is powered to detect the differences in the gut microbiota, antibiotic resistance, and metabolic parameters. Third, the antibiotic resistances of *E coli* and *K pneumoniae* to macrolides were not assessed. However, the antibiotics commonly used for Gram-negative rod infections were included in the analysis. Further studies are warranted to assess the changes of antibiotic resistance, including macrolides resistance, of other bacteria, such as staphylococcus and streptococcus. Fourth, long-term outcomes in untreated *H pylori* infected patients were not included as a control group in this study. However, the long-term outcomes of different regimens were compared in this randomised trial and the baseline status was used as a comparator. Besides, it would be difficult to randomly assign *H pylori* infected patients into the no eradication group because Consensus Reports recommend that eradication therapy should be offered to infected patients unless there are competing considerations.[31] Fifth, the dietary habits and medication history were not recorded during the follow-up periods. Yet, the underlying illness (eg, reflux esophagitis) and lifestyles were similarly distributed among the three treatment groups. Although some of them might change their dietary habits, the proportion is expected to be similar among the three randomly assigned groups. 5–10% of the study patients might take continuous PPI therapy owing to grade C–D erosive esophagitis, but the proportions of erosive esophagitis were similar in the three treatment groups. Finally, the changes in the species level after eradication therapy could not be reliably assessed through sequencing of 16S rRNA. Further in-depth sequencing, such as whole genome shotgun sequencing, would be needed.

In conclusion, although there were transient perturbation and changes in the gut microbiota immediately after *H pylori* eradication therapy, most of these changes resolved and returned to pretreatment levels by 8 weeks and certainly by 1 year. Yet, the speed and extent of restoration varied with regimens. Similarly, although the antibiotic resistance prevalence of *E coli* and *K pneumoniae* to certain antibiotics were transiently increased immediately after triple and concomitant therapies, the resistance prevalence returned to its pretreatment value at week 8 and 1 year. It is noteworthy that there were no significant increases in the antibiotic resistance rates of *E coli* after bismuth quadruple therapy. There were no significant changes in the prevalence of metabolic syndrome at week 8 and 1 year after eradication therapy. Although there were trivial increases in body-mass index and bodyweight, the insulin resistance and triglyceride concentrations decreased, indicating there are potential beneficial metabolic effects after *H pylori* eradication. These results collectively lend support to the long-term safety of *H pylori* eradication therapies.

Contributors

The study was conceived by J-ML with input from M-SWu and EME-O and all the other listed contributors from the Taiwan Gastrointestinal Disease and Helicobacter Consortium. J-M L designed the study and wrote the protocol. J-ML, Chieh-CC, Y-JF, M-JB, P-YC, C-YC, Y-CH, M-JC, Chien-CC, J-YL, T-HY, J-CL, C-YC, W-FH, WH Hu, Y-NC, J-YW, J-TL, and M-SW recruited patients to the study. J-ML prepared the statistical analyses. C-MC, T-PL, and EYC performed the bioinformatics analysis. J-ML drafted the article which was critically revised by two senior authors, M-SW and EME-O. All authors commented on drafts and approved the

final version. All authors had full access to the data and participated in the decision to submit for publication.

Taiwan Gastrointestinal Disease and Helicobacter Consortium investigators
Steering committee of The Taiwan Gastrointestinal Disease and Helicobacter Consortium: Jyh-Ming Liou (Taipei), Yi-Chia Lee (Taipei), Mei-Jyh Chen (Taipei), Jaw-Town Lin (Taipei), Chun-Ying Wu (Taipei), Jeng-Yih Wu (Kaohsiung), Chia-Tung Shun (Taipei), Chun-Hung Lin (Taipei), Yu-Ren Fang (Yun-Lin), Ming-Jong Bair (Taitung), Jiing-Chyuan Luo (Taipei), and Ming-Shiang Wu (Taipei).
Other investigators of the Taiwan Helicobacter Consortium in this study: Ping-Huei Tseng (Taipei), Han-Mo Chiu (Taipei), Chun-Chao Chang (Taipei), Chien-Chun Yu (Yun-Lin), Min-Chin Chiu (Yun-Lin), Yen-Nien Chen (Hsinchu), Wen-Hao Hu (Hsinchu), Chu-Kuang Chou (Chia-Yi), Chi-Ming Tai (Kaohsiung), and Wen-Shiung Chang (Taipei).

Declaration of interests
We declare no competing interests.

Data sharing
The study protocol and statistical analysis plan are published online. Appropriate academic parties may contact Jyh-Ming Liou (jyhmingliou@gmail.com) for the de-identified participant dataset that underlies the results reported in this article, in accordance with the data sharing policies of National Taiwan University and Ministry of Health and Wealth, Taiwan, with input from the investigator group where applicable after receipt of the research proposal.

Acknowledgments
The study was funded by the National Taiwan University Hospital (Grant Number: NTUH 104-P05, NTUH 105-P05, NTUH 106–P06, and NTUH 107-P05), the Ministry of Science and Technology, Executive Yuan, ROC, Taiwan (Grant Number: TCTC-TR2 106-2321-B-002-025 and MOST 107-3017-F-002-002), the Ministry of Health and Welfare of Taiwan (Grant Number: MOHW106-TDU-B-211-113002, MOHW107-TDU-B-211-123002), the "Center of Precision Medicine" from The Featured Areas Research Center Program within the framework of the Higher Education Sprout Project by the Ministry of Education (MOE) in Taiwan (Grant Number: NTU-107L9014-1), the Taipei Institute of Pathology (Grant Number: TIP-106-001 and TIP-107-005), and the Liver Disease Prevention & Treatment Research Foundation, Taiwan. EE-O is funded by grants from the Australian Federal Government to the St George and Sutherland Medical Research Foundation. The lansoprazole used in the present study were provided for free by the Takeda Company (Taiwan). The authors would like to express their special thanks to the Eighth Core Lab, Department of Medical Research, National Taiwan University Hospital, the Taipei Institute of Pathology, and the First Core Lab, Division of Microbiology, National Taiwan University College of Medicine, for their technological support. MS Wu and EM El-Omar shared the co-senior authorships in this work.

References
1 Malfertheiner P, Megraud F, O'Morain CA, et al. Management of *Helicobacter pylori* infection-the Maastricht V/Florence Consensus Report. *Gut* 2017; **66:** 6–30.
2 Choi IJ, Kook MC, Kim YI, et al. *Helicobacter pylori* therapy for the prevention of metachronous gastric cancer. *N Engl J Med* 2018; **378:** 1085–95.
3 Lee YC, Chiang TH, Chou CK, et al. Association between *Helicobacter pylori* eradication and gastric cancer incidence: a systematic review and meta-analysis. *Gastroenterology* 2016; **150:** 1113–24.
4 Graham DY. *Helicobacter pylori* update: gastric cancer, reliable therapy, and possible benefits. *Gastroenterology* 2015; **148:** 719–31.
5 Chey WD, Leontiadis GI, Howden CW, Moss SF. ACG Clinical guideline: treatment of *Helicobacter pylori* infection. *Am J Gastroenterol* 2017; **112:** 212–39.
6 Fallone CA, Chiba N, van Zanten SV, et al. The Toronto Consensus for the Treatment of *Helicobacter pylori* Infection in adults. *Gastroenterology* 2016; **151:** 51–69.
7 Liou JM, Fang YJ, Chen CC, et al. Concomitant, bismuth quadruple, and 14-day triple therapy in the first-line treatment of *Helicobacter pylori:* a multicentre, open-label, randomized trial. *Lancet* 2016; **388:** 2355–65.
8 O'Connor A, O'Morain CA, Ford AC. Population screening and treatment of *Helicobacter pylori* infection. *Nat Rev Gastroenterol Hepatol* 2017; **14:** 230–40.
9 Sjölund M, Wreiber K, Andersson DI, Blaser MJ, Engstrand L. Long-term persistence of resistant *Enterococcus* species after antibiotics to eradicate *Helicobacter pylori*. *Ann Intern Med* 2003; **139:** 483–87.
10 Jakobsson H, Wreiber K, Fall K, Fjelstad B, Nyrén O, Engstrand L. Macrolide resistance in the normal microbiota after *Helicobacter pylori* treatment. *Scand J Infect Dis* 2007; **39:** 757–63.
11 Blaser MJ. Antibiotic use and its consequences for the normal microbiome. *Science* 2016; **352:** 544–45.
12 Blaser M. Antibiotic overuse: stop the killing of beneficial bacteria. *Nature* 2011; **476:** 393–94.
13 Yap TW, Gan HM, Lee YP, et al. *Helicobacter pylori* eradication causes perturbation of the human gut microbiome in young adults. *PLoS One* 2016; **11:** e0151893.
14 Jakobsson HE, Jernberg C, Andersson AF, Sjölund-Karlsson M, Jansson JK, Engstrand L. Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. *PLoS One* 2010; **5:** e9836.
15 Hsu PI, Pan CY, Kao JY, et al. *Helicobacter pylori* eradication with bismuth quadruple therapy leads to dysbiosis of gut microbiota with an increased relative abundance of Proteobacteria and decreased relative abundances of Bacteroidetes and Actinobacteria. *Helicobacter* 2018; **23:** e12498.
16 Yanagi H, Tsuda A, Matsushima M, et al. Changes in the gut microbiota composition and the plasma ghrelin level in patients with *Helicobacter pylori*-infected patients with eradication therapy. *BMJ Open Gastroenterol* 2017; **4:** e000182.
17 Polyzos SA, Kountouras J, Zavos C, Deretzi G. The association between *Helicobacter pylori* infection and insulin resistance: a systematic review. *Helicobacter* 2011; **16:** 79–88.
18 Upala S, Jaruvongvanich V, Riangwiwat T, Jaruvongvanich S, Sanguankeo A. Association between *Helicobacter pylori* infection and metabolic syndrome: a systematic review and meta-analysis. *J Dig Dis* 2016; **17:** 433–40.
19 Upala S, Sanguankeo A, Saleem SA, Jaruvongvanich V. Effects of *Helicobacter pylori* eradication on insulin resistance and metabolic parameters: a systematic review and meta-analysis. *Eur J Gastroenterol Hepatol* 2017; **29:** 153–59.
20 Kountouras J, Polyzos SA, Doulberis M, et al. Potential impact of *Helicobacter pylori*-related metabolic syndrome on upper and lower gastrointestinal tract oncogenesis. *Metabolism* 2018; **87:** 18–27.
21 Sonnenburg JL, Bäckhed F. Diet-microbiota interactions as moderators of human metabolism. *Nature* 2016; **535:** 56–64.
22 Lynch SV, Pedersen O. The human intestinal microbiome in health and disease. *N Engl J Med* 2016; **375:** 2369–79.
23 Tang WH, Wang Z, Levison BS, et al. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *N Engl J Med* 2013; **368:** 1575–84.
24 Ianiro G, Tilg H, Gasbarrini A. Antibiotics as deep modulators of gut microbiota: between good and evil. *Gut* 2016; **65:** 1906–15.
25 Korpela K, Salonen A, Virta LJ, et al. Intestinal microbiome is related to lifetime antibiotic use in Finnish pre-school children. *Nat Commun* 2016; **7:** 10410.
26 American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2014; **37** (suppl 1)**:** S81–90.
27 Oh B, Kim BS, Kim JW, et al. The effect of probiotics on gut microbiota during the *Helicobacter pylori* eradication: randomized controlled trial. *Helicobacter* 2016; **21:** 165–74.
28 Chen L, Xu W, Lee A, et al. The impact of *Helicobacter pylori* infection, eradication therapy and probiotic supplementation on gut microenvironment homeostasis: an open-label, randomized clinical trial. *EBioMedicine* 2018; **35:** 87–96.
29 MacPherson CW, Mathieu O, Tremblay J, et al. Gut bacterial microbiota and its resistome rapidly recover to basal state levels after short-term amoxicillin-clavulanic acid treatment in healthy adults. *Sci Rep* 2018; **8:** 11192.
30 Lane JA, Murray LJ, Harvey IM, Donovan JL, Nair P, Harvey RF. Randomised clinical trial: *Helicobacter pylori* eradication is associated with a significantly increased body mass index in a placebo-controlled study. *Aliment Pharmacol Ther* 2011; **33:** 922–29.
31 Sugano K, Tack J, Kuipers EJ, et al. Kyoto global consensus report on *Helicobacter pylori* gastritis. *Gut* 2015; **64:** 1353–67.