Sequential versus triple therapy for the first-line treatment of *Helicobacter pylori*: a multicentre, open-label, randomised trial

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Summary

Background Whether sequential treatment can replace triple therapy as the standard treatment for *Helicobacter pylori* infection is unknown. We compared the efficacy of sequential treatment for 10 days and 14 days with triple therapy for 14 days in first-line treatment.

Methods For this multicentre, open-label, randomised trial, we recruited patients (≥20 years of age) with *H pylori* infection from six centres in Taiwan. Using a computer-generated randomisation sequence, we randomly allocated patients (1:1:1; block sizes of six) to either sequential treatment (lansoprazole 30 mg and amoxicillin 1 g for the first 7 days, followed by lansoprazole 30 mg, clarithromycin 500 mg, and metronidazole 500 mg for another 7 days; with all drugs given twice daily) for either 10 days (S-10) or 14 days (S-14), of 14 days of triple therapy (T-14; lansoprazole 30 mg, amoxicillin 1 g, and clarithromycin 500 mg for 14 days; with all drugs given twice daily). Investigators were masked to treatment allocation. Our primary outcome was the eradication rate in first-line treatment by intention-to-treat (ITT) and per-protocol (PP) analyses. This trial is registered with ClinicalTrials.gov, number NCT01042184.

Findings Between Dec 28, 2009, and Sept 24, 2011, we enrolled 900 patients: 300 to each group. The eradication rate was 90·7% (95% CI 87·4–94·0; 272 of 300 patients) in the S-14 group, 87·0% (83·2–90·8; 261 of 300 patients) in the S-10 group, and 82·3% (78·0–86·6; 247 of 300 patients) in the T-14 group. Treatment efficacy was better in the S-14 group than it was in the T-14 group in both the ITT analysis (number needed to treat of 12·0 [95% CI 7·2–34·5]; p=0·003) and PP analyses (13·7 [8·3–40], p=0·003). We recorded no significant difference in the occurrence of adverse effects or in compliance between the three groups.

Interpretation Our findings lend support to the use of sequential treatment as the standard first-line treatment for *H pylori* infection.

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Introduction

Helicobacter pylori is an important cause of peptic ulcer disease and gastric cancer, but eradication rates with standard triple therapy have decreased to less than 80% in many countries.[1–5] Several strategies have been proposed to increase the eradication rate, including the extending of treatment duration to 14 days, the use of a four-drug regimen (quadruple, sequential, and concomitant treatments), and the use of novel antibiotics such as levofloxacin.[6–13] Sequential treatment, which consists of a proton-pump inhibitor and amoxicillin for the first 5 days, followed by a proton-pump inhibitor plus clarithromycin and metronidazole (or tinidazole) for another 5 days, has been shown to be more effective than triple therapy for 7 days or 10 days.[11–13] The efficacy of sequential treatment seemed to be affected less by clarithromycin resistance than is triple therapy and has the potential to become the standard first-line treatment for *H pylori* infection.[15,16]

However, several concerns need to be resolved before sequential treatment can replace triple therapy as the standard treatment.[15,16] First, most of the studies did not do susceptibility tests and their results cannot be generalised to other countries where the prevalence of antibiotic resistance is different. Second, few studies compared sequential treatment with triple therapy for 14 days, which is recommended by US guidelines.[4,15] Two studies from Latin America and South Korea that compared sequential treatment for 10 days with triple therapy for 14 days, however, showed contradictory results.[9,17] The reasons behind the contradictory results were unknown because susceptibility tests were not done.[9,17,18] Third, whether extending the duration of sequential treatment from 10 days to 14 days would be more effective than triple therapy for 14 days is unknown. Fourth, despite the fact that knowing how to re-treat patients who fail sequential treatment is important, few studies addressed this issue.[19] Finally, how to choose the best regimen on the basis of the prevalence of antibiotic resistance in different geographical areas is unknown.

To address these issues, we did a randomised controlled trial to compare the efficacy of sequential treatment for 10 days and 14 days with triple therapy for 14 days in first-line treatment. We extensively assessed factors that might affect eradication rates, such as antibiotic resistance, host CYP2C19 polymorphisms, and bacterial virulence factors (CagA and VacA). We also assessed the

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efficacy of the modified sequential treatment containing levofloxacin in patients who failed sequential treatment and triple therapy.[20] We constructed a decision model to estimate the efficacies of three regimens in the sensitivity analysis according to the prevalence of antibiotic resistance, aiming to solve the heterogeneity of treatment efficacies seen in previous studies.

Methods

Study design and participants

For this multicentre, open-label, randomised trial, we recruited participants from gastroenterology clinics in six medical centres in Taiwan. Study staff recruited potential participants and explained to them the purpose of the trial and eligibility requirements for enrolment. Patients were eligible for recruitment if they were aged 20 years or older and had documented *H pylori* infection. Patients with any one of the following criteria were excluded from the study: previous eradication treatment for *H pylori*, history of gastrectomy, contraindication or previous allergic reactions to the study drugs, pregnant or lactating women, use of antibiotics within the previous 4 weeks, and severe concurrent diseases or malignancy.

Participants provided written informed consent before enrolment. This trial was approved by the Institutional Review Board of each hospital.

Randomisation and masking

Using a permuted block randomisation with a block size of six, we randomly allocated eligible patients to receive one of the following regimens (1:1:1): sequential treatment for 14 days (S-14), sequential treatment for 10 days (S-10), or triple therapy for 14 days (T-14; all given twice daily). An independent research assistant at the National Taiwan University Hospital generated the computerised random number sequence. The sequence was concealed in an opaque envelope until the intervention was assigned. Envelopes were kept at the National Taiwan University Hospital. After the written informed consents were obtained from eligible patients, the independent research assistant telephoned study staff to give them each patient's treatment allocation. All investigators were masked to the randomisation sequence. Patients who

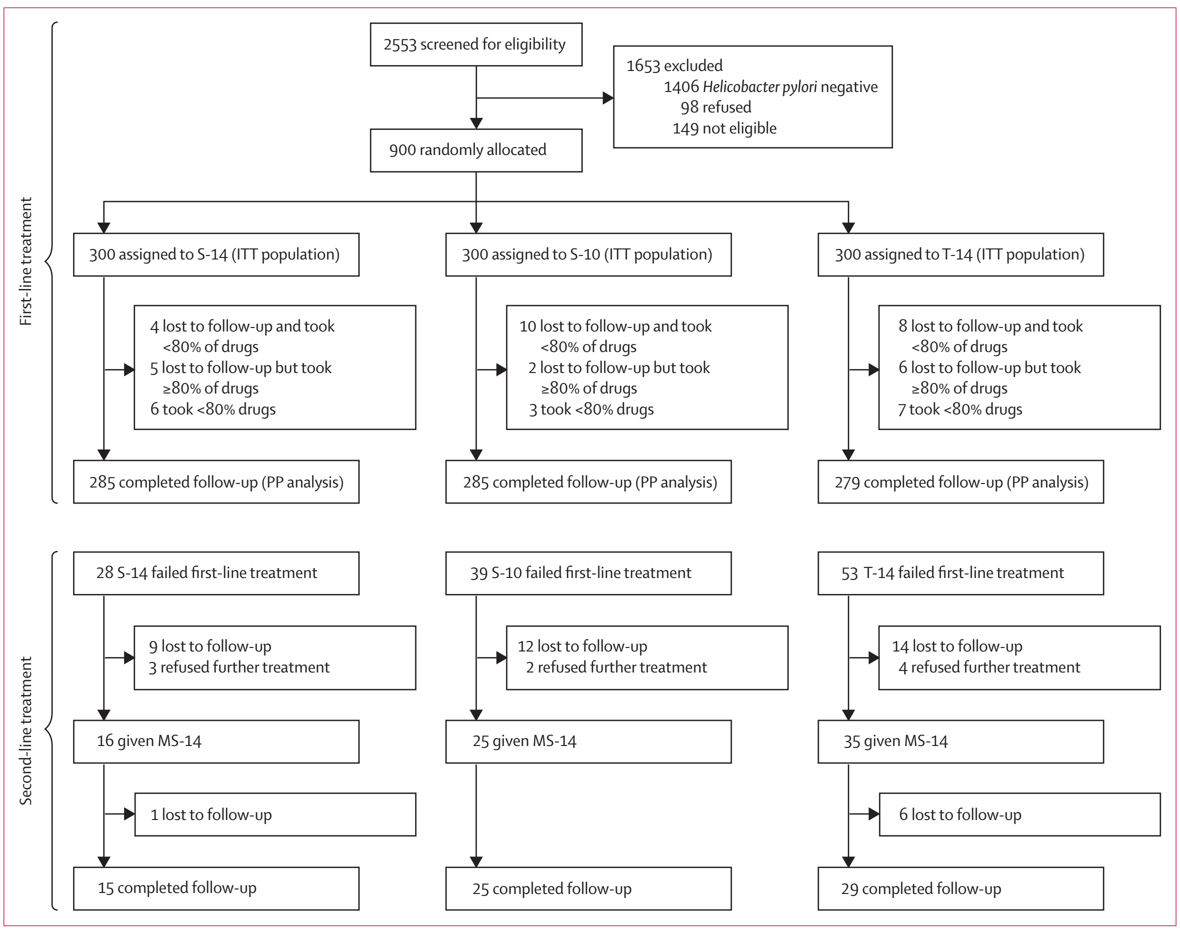

***Figure 1:* Trial profile**

ITT=intention-to-treat. PP=per-protocol. MS-14=modified sequential treatment containing levofloxacin. S-10=sequential treatment for 10 days. S-14=sequential treatment for 14 days. T14=triple therapy for 14 days.

remained positive for *H pylori* after the initial treatment were retreated with modified sequential treatment for 14 days (MS-14).

Procedures

Study treatment regimens were all given twice a day and contained the following: S-14 (lansoprazole 30 mg and amoxicillin 1 g for the first 7 days, followed by lansoprazole 30 mg, clarithromycin 500 mg, and metronidazole 500 mg for another 7 days), S-10 (lansoprazole 30 mg and amoxicillin 1 g for the first 5 days, followed by lansoprazole 30 mg, clarithromycin 500 mg, and metronidazole 500 mg for another 5 days), T-14 (lansoprazole 30 mg, amoxicillin 1 g, and clarithromycin 500 mg for 14 days). MS-14 was also given twice a day and contained lansoprazole 30 mg and amoxicillin 1 g for the first 7 days, followed by lansoprazole 30 mg, metronidazole 500 mg, and levofloxacin 250 mg for another 7 days.

Before enrolment, the status of *H pylori* infection in patients with upper gastrointestinal symptoms was determined by rapid urease test, histology, culture, and serology. Patients with positive results in at least two of these tests were eligible for enrolment. Asymptomatic individuals who underwent cancer screening were also eligible for enrolment if they had a positive ^{13}C urea breath test (^{13}C-UBT). Post-treatment *H pylori* status was assessed by ^{13}C-UBT at least 6 weeks after completion of treatment. All patients were asked to stop treatment with proton-pump inhibitor and histamine-2 blocker for at least 2 weeks before their ^{13}C-UBT. The urea kit (which contained 75 mg ^{13}C-urea) was dissolved in water and mixed with orange juice. Baseline and 30 min breath samples were assayed with an infrared spectrometer that produced computer-generated results in the Taipei Institute of Pathology (Taipei City, Taiwan). Positive and negative results were defined according to results of our previous validation study[21] as a Δ value of 4 units or higher for positive and less than 2·5 units for negative. Patients with inconclusive results received another ^{13}C-UBT at least 2 weeks after the inconclusive test until the results were conclusive.

The primary endpoint of the study was *H pylori* eradication rates in first-line treatment. The secondary endpoints were the frequency of adverse events and treatment compliance. The patients were informed of the common side-effects from the study drugs before treatment and were asked to record these symptoms during treatment in provided diaries. A standardised interview was also arranged at the end of treatment to assess the adverse events and compliance. Compliance was recorded as low when less than 80% of pills were taken.

The biopsy specimens were cultured on plates containing Brucella chocolate agar with 7% sheep blood and incubated for 7 days under microaerobic conditions. The minimum inhibitory concentrations were assessed by agar dilution test in the central laboratory in National Taiwan University Hospital. We defined resistance breakpoints for every antibiotic (amoxicillin ≥0·5 mg/L, clarithromycin ≥1 mg/L, levofloxacin ≥1 mg/L, and metronidazole ≥8 mg/L).[8,22] The genotypes of *gyr*A and 23S rRNA were established by PCR followed by direct sequencing with the automatic sequencer (ABI PRISM 3100 Genetic Analyzer; Applied Biosystems, Foster City, CA, USA).[22] The CagA gene and the VacA signal region (signal region 1 and 2) and midregion (midregion 1 and 2) mosaics were determined by PCR as described previously.[23] Genotyping for the CYP2C19 polymorphism was done with the SEQUENOM MassARRAY System (Sequenom, Sand Diego, CA, USA) in the Taiwan National Genotyping Centre.[24]

Statistical analysis

On the basis of findings from a previous meta-analysis,[15] we hypothesised that there would be about a 10% difference in the eradication rates between the three study regimens. Findings from a previous study suggested that the eradication rate with T-14 would be about 85%,[25] so our original sample size estimation was for at least 155 individuals in each group, giving a power of 80% and a 0·05 two-sided type 1 error, assuming 10% loss to follow-up. After an interim report, we decided to increase the sample size to a conservative estimate of 300 individuals in each group, which would give a power of 90% in rejecting the null hypothesis and to adjust the type 1 error for multiple comparisons with Bonferroni correction. We made this decision to increase the

	S-14 group (N=300)	S-10 group (N=300)	T-14 group (N=300)
Men	165 (55%)	159 (53%)	167 (56%)
Mean age in years (SD)	53·7 (12·5)	52·8 (13·8)	53·3 (14·1)
Cigarette smoking	59 (20%)	68 (23%)	67 (22%)
Alcohol drinking (>40mL)	77 (26%)	71 (24%)	74 (25%)
Peptic ulcer disease	193 (64%)	209 (70%)	197 (66%)
Body-mass index of 27 or greater	63 (21%)	52 (17%)	66 (22%)
CYP2C19 (poor metaboliser)	43/286 (15%)	27/286 (9%)	38/287 (13%)
CagA-positive	147/177 (83%)	157/191 (82%)	144/183 (79%)
23S *rRNA* mutation	15/178 (8%)	15/192 (8%)	21/183 (11%)
GyrA mutation	16/172 (9%)	23/190 (12%)	17/179 (9%)
Clarithromycin resistance	16/177 (9%)	18/192 (9%)	21/183 (11%)
Metronidazole resistance	39/177 (22%)	46/192 (24%)	48/183 (26%)
Amoxicillin resistance	4/177 (2%)	4/192 (2%)	5/183 (3%)
Levofloxacin resistance	17/177 (10%)	22/192 (11%)	22/183 (12%)
Helicobacter pylori test			
Serology	293/298 (98%)	292/295 (99%)	291/298 (98%)
Rapid urease test	235/253 (93%)	237/255 (93%)	239/252 (95%)
Histology	252/263 (96%)	254/264 (96%)	248/262 (95%)
Culture	181/234 (77%)	195/235 (83%)	184/230 (80%)
Urea breath test	66/66 (100%)	65/65 (100%)	70/70 (100%)

Data are number of patients (%) or patients positive/patients tested (%), unless otherwise stated. S-10=sequential treatment for 10 days. S-14=sequential treatment for 14 days. T-14=triple therapy for 14 days.

Table 1: **Baseline characteristics**

	S-14 group	S-10 group	T-14 group	p value
Eradication after first-line treatment				
ITT analysis (n/N [%; 95% CI])	272/300 (90·7%; 87·4–94·0)*	261/300 (87·0%; 83·2–90·8)	247/300 (82·3%; 78·0–86·6)*	0·011
PP analysis (n/N [%; 95% CI])	269/285 (94·4%; 91·7–97·1)†‡	258/285 (90·5%; 87·1–93·9)‡	243/279 (87·1%; 83·2–91·0)†‡	0·012
Eradication after second-line treatment				
ITT analysis (n/N [%; 95% CI])	283/300 (94·3%; 91·7–97·0)	283/300 (94·3%; 91·7–97·0)	275/300 (91·7%; 88·6–94·8)	0·31
PP analysis (n/N [%; 95% CI])	280/284 (98·6%; 96·4–99·4)	280/285 (98·2%; 96·0–99·2)	271/273 (99·3%; 97·4–100)	0·56

ITT=intention-to-treat. PP=per-protocol. S-10=sequential treatment for 10 days. S-14=sequential treatment for 14 days. T-14=triple therapy for 14 days. *p=0·003 for S-14 *vs* T-14. †p=0·003 for S-14 *vs* T-14. ‡In the six patients in the S-14 group, three patients in the S-10 group, and seven patients in the T-14 group who took less than 80% of the study drugs, *H pylori* eradication was successfully achieved in three patients, three patients, and four patients, respectively.

Table 2:* *Helicobacter pylori **eradication in first-line and second-line treatments**

For the **online calculator** see http://hp-therapy.biomed.org.tw

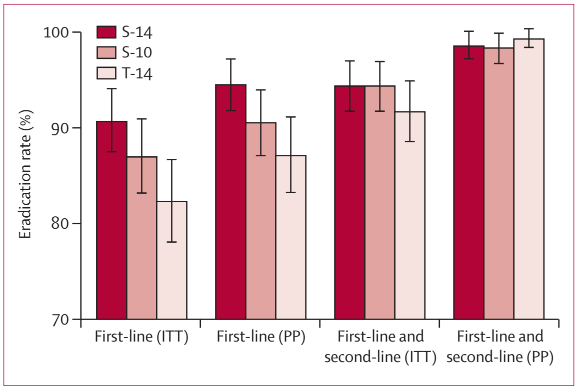

Figure 2: **Efficacies of first-line and second-line *Helicobacter pylori* treatments**
ITT=intention to treat. PP=per protocol. MS-14=modified sequential treatment containing levofloxacin. S-10=sequential treatment for 10 days. S-14=sequential treatment for 14 days. T14=triple therapy for 14 days. Absolute differences in the efficacy of first-line and second-line treatments are given in the appendix.

See **Online** for appendix

precision of our study and to ensure an overall nominal significance level of 0·05, assuming 15% loss to follow-up. We did intention-to-treat (ITT) and per-protocol (PP) analyses in the assessment of the primary endpoint. All randomised patients were included in the ITT analysis. All individuals who violated the study protocol, such as patients not taking at least 80% of treatment drugs, or with unknown post-treatment *H pylori* status were excluded from the PP analysis. Patients who did not return for a follow-up ^{13}C-UBT were recorded as treatment failures. We compared categorical data using the χ^2 test or Fisher's exact test, as appropriate. We compared continuous data with the Student's *t* test and gave results as mean (SD). For the primary endpoint, we adjusted for multiple comparisons by setting a Bonferroni-corrected α level of 0·01. For secondary variables, we did exploratory analyses by setting an α level of 0·05 without adjustment for multiple comparisons. We used SPSS (version 12.0 for Microsift Windows) for all statistical analyses.

To assess factors affecting eradication rates, we did a multiple logistic regression analyses with the following predictors of interest: clarithromycin resistance, metronidazole resistance, amoxicillin resistance, age, sex, peptic ulcer disease, and smoking. We did not include patients with missing data in the regression analyses. After identification of factors associated with treatment failure, we constructed a decision model (not described in the protocol) to elucidate the heterogeneity of treatment efficacy seen in previous studies (the decision model is available in the form of an online calculator). We did deterministic and probabilistic sensitivity analyses to investigate the effects of changes in the prevalence of the antibiotic resistant strains across a wide range of assumptions. We did a decision model analysis using a commercially available software package (TreeAge Pro 2009; version 1.0.2).

This study is registered with ClinicalTrial.gov, number NCT01042184.

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Dec 28, 2009, and Sept 24, 2011, we enrolled 900 patients (figure 1). Biopsy for culture was done for 699 patients who underwent endoscopy because of upper gastrointestinal symptoms but not for the other 201 participants who underwent cancer screening. Drug susceptibility data were available in 552 patients. Baseline characteristics were much the same across the three groups (table 1). The mean interval between completion of treatment and the follow-up ^{13}C-UBT were 7·29 weeks (SD 1·61) in the S-14 group, 7·17 weeks (1·50) in the S-10 group, and 7·14 weeks (1·49) in the T-14 group.

H pylori eradication was greater in the S-14 group than it was in the T-14 group in both the ITT (number needed to treat 12 [95% CI 7·2–34·5]; p=0·003) and PP analyses (number needed to treat 13·7 [8·3–40·0]; p=0·003; table 2 and figure 2). We recorded no statistically significant difference in treatment efficacy between the S-14 group and the S-10 group or between the S-10 group and the T-14 group (figure 2). *H pylori* eradication in patients who received MS-14 after failing first-line treatment was

80·5% (95% CI 66–89·9; 33 of 41 patients) in those who had received sequential treatment and 80·0% (64·1–90·0; 28 of 35 patients) in those who had received triple therapy. We recorded no significant difference in the overall eradication rates after two courses of antibiotic treatment between any of the three groups.

We recoded no statistically significant difference in the occurrence of adverse effects or in compliance between the three groups, or in patients rescued with MS-14 between the three treatment groups (table 3).

The eradication rates of S-14, S-10, and T-14 therapies were all affected by clarithromycin resistance (table 4). These findings were consistent with different methods to detect the clarithromycin resistance (genotypic and phenotypic resistance). *H pylori* eradication in the S-14 and S-10 groups were also affected by the presence of metronidazole resistance. In strains susceptible to both clarithromycin and metronidazole, the eradication rate was higher in patients treated with S-14 than those treated with T-14 (p=0·006; table 4). The eradication rates of S-14, S-10, and T-14 therapies were also affected by compliance, but not by host CYP2C19 polymorphism or bacterial virulence factors. 552 patients with drug susceptibility data were included in the multiple logistic regression analyses. We recorded no statistically significant difference in eradication rates between the groups with or without drug susceptibility data (appendix). Multiple regression analyses showed that clarithromycin resistance was associated with treatment failure in all three groups; metronidazole resistance was associated with treatment failure in the S-14 and S-10 groups (table 4). Although amoxicillin resistance was also associated with treatment failure in the S-14 and T-14 groups, the occurrence of amoxicillin resistance was very rare (<3% of all participants) and so this finding should be interpreted with caution.

With the knowledge that clarithromycin resistance and metronidazole resistance were the main determinants for treatment failure, we constructed a decision model based on these two factors (see appendix for information about the model structure, input parameters, and the model credibility). Deterministic sensitivity analyses showed that the efficacies of S-14, S-10, and T-14 decreased with increasing prevalences of clarithromycin resistance (appendix). S-14 was the most efficacious regimen in all global regions, except in areas with very low (<5%) clarithromycin resistance and very high (>80%) metronidazole resistance. S-10 seemed to be more effective than T-14 only in areas where metronidazole resistance was lower than 40%. Probability sensitivity analyses consistently showed that T-14 was a poor choice for treatment in most of Taiwan.

	S-14 group	S-10 group	T-14 group	p value
First-line treatment				
Dizziness	34/300 (11%)	31/295 (11%)	19/299 (6%)	0·26
Skin rash	7/300 (2%)	9/295 (3%)	7/299 (2%)	0·31
Headache	15/300 (5%)	9/295 (3%)	16/299 (5%)	0·70
Taste distortion	63/300 (21%)	58/295 (20%)	76/299 (25%)	0·51
Abdominal pain	28/300 (9%)	19/295 (6%)	31/299 (10%)	0·39
Nausea	24/300 (8%)	23/295 (8%)	11/299 (4%)	0·25
Diarrhoea	39/300 (13%)	48/295 (16%)	62/299 (21%)	0·23
Constipation	7/300 (2%)	9/295 (3%)	11/299 (4%)	0·63
Bloating	23/300 (8%)	21/295 (7%)	17/299 (6%)	0·67
Any adverse events	161/299 (54%)	142/294 (48%)	164/298 (55%)	0·22
Discontinued drugs because of adverse events	14/299 (5%)	6/295 (2%)	13/297 (4%)	0·39
Took at least 80% of drugs	290/300 (97%)	287/295 (97%)	285/299 (95%)	0·52
Took the drugs correctly	287/300 (96%)	280/295 (95%)	277/299 (93%)	0·37
Second-line treatment with modified sequential treatment containing levofloxacin				
Dizziness	4/16 (25%)	2/25 (8%)	5/33 (15%)	0·49
Skin rash	0/10	0/25	1/33 (3%)	0·53
Headache	1/16 (6%)	0/25 (0%)	0/33 (0%)	0·16
Taste distortion	0/16 (0%)	3/25 (12%)	4/33 (12%)	0·52
Abdominal pain	0/16 (0%)	2/25 (8%)	1/33 (3%)	0·62
Nausea	1/16 (6%)	5/25 (20%)	3/33 (9%)	0·12
Diarrhoea	1/16 (6%)	2/25 (8%)	6/33 (18%)	0·63
Constipation	1/16 (6%)	0/25 (0%)	2/33 (6%)	0·45
Bloating	2/16 (13%)	1/25 (4%)	2/33 (6%)	0·30
Any adverse events	6/16 (38%)	13/25 (52%)	16/33 (48%)	0·65
Discontinued drugs because of adverse events	0/16 (0%)	0/25 (0%)	0/33 (0%)	..
Took at least 80% of drugs	16/16 (100%)	25/25 (100%)	29/33 (88%)	0·07
Took the drugs correctly	15/16 (94%)	25/25 (100%)	28/33 (85%)	0·11

Data are n/N (%). S-10=sequential treatment for 10 days. S-14=sequential treatment for 14 days. T-14=triple therapy for 14 days.

Table 3: **Adverse events in first-line and second-line treatment**

Discussion

Our study had several novel findings. First, we know of no other study to show that sequential treatment for 14 days is better than triple therapy for 14 days as first-line treatment. Second, by thoroughly assessing antibiotic susceptibility, we detected that clarithromycin resistance decreased the efficacies of both sequential and triple treatments, and that metronidazole resistance decreased the efficacy of sequential treatment.[9,11,12,17,26] Third, our findings suggest that *H pylori* eradication rates with the three studied regimens are not affected by host CYP2C19 polymorphisms nor bacterial virulence factors, which have been reported to be associated with treatment failure in patients receiving triple therapy for 7 days or 10 days.[27–29] Fourth, our findings suggest that modified sequential treatment containing levofloxacin is effective for patients who failed from either sequential or triple therapy. Taken together, our findings lend support to the use of sequential treatment as an alternative to triple therapy for first-line treatment of patients with *H pylori* infection.

Of the two randomised trials that compared the effect of clarithromycin resistance on the eradication of sequential and triple therapies, Zullo and colleagues[12] showed that eradication with sequential treatment was

	S-14 group	S-10 group	T-14 group
Univariate analyses			
23S rRNA mutation (genotypic)			
No	148/153 (97%)	154/169 (91%)	136/151 (90%)
Yes	9/13 (69%)	9/14 (64%)	12/20 (60%)
Clarithromycin resistance (phenotypic)			
Susceptible	146/150 (97%)	152/166 (92%)	137/151 (91%)
Resistant	10/15 (67%)	10/17 (59%)	11/20 (55%)
Metronidazole resistance (phenotypic)			
Susceptible	126/131 (96%)	130/139 (94%)	107/125 (86%)
Resistant	30/34 (88%)	32/44 (73%)	41/46 (89%)
Amoxicillin resistance (phenotypic)			
Susceptible	154/161 (96%)	160/179 (89%)	147/166 (89%)
Resistant	2/4 (50%)	2/4 (50%)	1/5 (20%)
Clarithromycin (Cla) and metronidazole (Met) resistance (phenotypic)			
Cla-S and Met-S	116/117 (99%)*	123/129 (95%)	98/109 (90%)*
Cla-S and Met-R	30/33 (91%)	29/37 (78%)	39/42 (93%)
Cla-R and Met-S	10/14 (71%)	7/10 (70%)	9/16 (56%)
Cla-R and Met-R	0/1	3/7 (43%)	2/4 (50%)
Compliance (took at least 80% of the drugs)			
Yes	269/285 (94%)	258/285 (91%)	243/278 (87%)
No†	3/6 (50%)	3/3 (100%)	4/7 (57%)
Peptic ulcer disease			
Yes	175/184 (95%)	180/199 (90%)	161/179 (90%)
No	94/101 (93%)	78/86 (91%)	82/100 (82%)
CYP2C19 polymorphism			
Poor metaboliser	40/42 (95%)	23/26 (89%)	31/36 (86%)
IM/EM	221/234 (94%)	228/252 (91%)	202/231 (87%)
CagA			
Positive	129/137 (94%)	134/149 (90%)	115/134 (86%)
Negative	28/28 (100%)	28/33 (85%)	33/37 (89%)
VacA			
Midregion 1	46/49 (94%)	46/52 (88%)	52/59 (88%)
Midregion 2	96/100 (96%)	100/114 (88%)	86/99 (87%)
Multivariate analyses‡			
Clarithromycin (resistance *vs* no resistance)	51·0 (4·67–559·24); p=0·0013	7·26 (2·05–25·70); p=0·002	12·1 (3·54–41·10); p<0·0001
Metronidazole (resistance *vs* no resistance)	20·7 (1·84–232·73); p=0·014	4·2 (1·50–11·72); p=0·006	0·6 (0·19–2·11); p=0·41
Amoxicillin (resistance *vs* no resistance)	32·7 (1·13–943·52); p=0·042	5·8 (0·46–72·81); p=0·17	39·6 (3·60–435·25); p=0·003

Data for univariate analysis are n/N (%) and data for multivariate analysis are adjusted odds ratio (95% CI); p value. S-10=sequential treatment for 10 days. S-14=sequential treatment for 14 days. T-14=triple therapy for 14 days. S=susceptible. R=resistant. EM=extensive metaboliser. IM=intermediate metaboliser. *p=0·006 for S-14 *vs* T-14. †Patients who did not take at least 80% of drugs but had returned for urea breath test were included. ‡The number of patients available for analysis in each group was as follows: 177 of 300 patients in the S-14 group, 192 of 300 patients in the S-10 group, and 183 of 300 patients in the T-14 group.

Table 4: **Factors affecting eradication in first-line treatment**

not affected by clarithromycin or metronidazole resistance, except in the presence of dual antibiotic resistance (panel).[11–14,26] Our findings suggest that when an *H pylori* strain was susceptible to both clarithromycin and metronidazole, S-14 was more effective than T-14. By contrast with their results, our results showed that eradication rates with S-10 and S-14 were also affected by resistance to both clarithromycin and metronidazole.[12,26] Possible explanations for the discrepancies included different nitroimidazole use in sequential treatment, different treatment duration of triple therapy, and differences in the ethnic origin of patients. However, we cannot exclude the possibility that the discrepancy between our findings and previous results might be caused by chance, because the numbers of patients with clarithromycin resistance in our study (n=32) and in a previous meta-analysis[26] (n=18) were small.

Although most of the clinical trials from Italy[11–13] showed that sequential treatment was more effective than triple therapy, results from Latin America[9] showed that sequential treatment was not better than triple therapy.[12,13,26] Our sensitivity analysis suggested that difference in the prevalence of antibiotic resistance between the groups was probably the most important explanation. When the reported prevalence of clarithromycin and metronidazole resistance of 24% and 80%[30] and 3·8% and 82%[31] in Latin America were applied in our model, we noted that T-14 seemed to be better than S-10 in terms of our two-way sensitivity analyses (appendix). Our study suggested that sequential treatment for 14 days was recommended in areas where the prevalence of clarithromycin resistance was less than 40%, especially when the prevalence of metronidazole resistance was greater than 40%. However, the duration of sequential treatment could be shortened to 10 days if the prevalence of metronidazole resistance is lower than 40%. In areas where clarithromycin resistance was greater than 40%, alternative treatments are recommended because neither sequential nor triple therapies achieved acceptable eradication rates (>80%). However, published data for antibiotic resistance prevalence for various countries should be interpreted with caution because almost all reports were based on highly selected groups of patients seen in urban referral facilities and because available antibiotic resistance data might not be readily generalisable. Therefore, the statistical inferences based on the antibiotic resistance results in our sensitivity analysis might be restricted.

The strength of this study included its large sample size, comparison of three treatment groups, extensive analysis of factors that might affect treatment efficacy, and assessment of the efficacy of their rescue treatment. A sensitivity analysis according to the prevalence of antibiotic resistance further solidified the generalisability of our findings. Therefore, our findings are expected to be useful in finding out the best treatment strategy according to the local prevalence of antibiotic resistance in different regions. The model constructed in our study would be useful in future clinical practice because of the dynamic change of antibiotic resistance over time and difficulties in doing further large-scale studies targeting treatment of *H pylori* infection as the prevalence of *H pylori* decreases with time.

Our study has limitations. First, antibiotic susceptibility data were available in only 61% of patients, which might raise the possibility of selection bias. This percentage was mainly related to the enrolment of individuals after cancer screening and also related to the fact that the culture rate of *H pylori* is less than perfect.[32] Individuals recruited on the basis of only one ^{13}C-UBT might also raise the possibility that some of them (about 4%) might not have had *H pylori* infection. However, through a randomised process, the proportions of patients recruited on the basis of their ^{13}C-UBT result were similar across three treatment groups, so their relative difference in treatment efficacy is unlikely to be affected. Furthermore, the treatment efficacies were indeed similar between the groups with and without antibiotic resistance data, so we believe that selection bias is unlikely. Second, although the actual difference between S-14 and T-14 in our study did not reach the presumed 10% difference, our sample size estimation was conservative and our findings, which indicated that S-14 was better than T-14, were of adequate power and the number needed to treat of 12 could be used as a measure for therapeutic decision making.[5] Nonetheless, this study was not powered to detect the difference in the overall efficacy after first-line and second-line treatment, and the precision in the efficacy estimate of MS-14 was constrained. Further studies are needed to assess the optimum algorithm for *H pylori* treatment. Third, this study was open label. Although we recorded no sunstantial difference between the baseline characteristics of the ITT and PP study population, patients who are lost to follow up or non-compliant might be as a direct result of their treatment allocation so that the PP population had higher eradication rates for each group and a smaller difference between the groups compared with the ITT population. Also, the complexity of sequential treatment might reduce patients' compliance outside clinical trials. Future studies are needed to assess whether the high eradication rates and adherence to treatment seen in this trial could be replicated in real-life practise. Fourth, the use of envelopes

Panel: Research in context

Systemic review

To compare the efficacy and the optimum treatment duration between sequential treatment and triple therapy, we searched PubMed for studies published between Jan 01, 2000, and Dec 31, 2011. Search terms included "*Helicobacter pylori* (*H pylori*)" and "sequential therapy" and "triple therapy". When the search was limited to randomised controlled trials published in English, we identified 18 trials that compared the efficacy of sequential treatment for 10 days versus triple therapy for 7 days or 10 days.[9–15] We identified no publications of clinical trials that compared 14-day sequential, 10-day sequential, and 14-day triple therapies for *H pylori* infection. None of the previous studies compared the efficacy of sequential treatment and triple therapy with sensitivity analysis according to the prevalence of clarithromycin and metronidazole resistance within a randomised trial.

Interpretation

Findings from our clinical trial suggest that sequential treatment for 14 days is more effective than triple therapy for 14 days in the first-line treatment of *H pylori* infection in an area with a prevalence of clarithromycin resistance of about 10% and metronidazole resistance of about 24%. In our decision model analysis, sequential treatment for either 10 days or 14 days was more efficacious than triple therapy for 14 days in all regions, except in areas with concomitantly high metronidazole and low clarithromycin resistance. Our results lend further support to the use of sequential treatment as the standard treatment in the first-line treatment of *H pylori* infection.

for randomisation might not have guaranteed adequate allocation concealment. However, in our study, the opaque envelopes were kept by one independent person and all investigators were masked to the randomisation sequence. The demographic characteristics and antibiotic resistance were similar among the three groups, which indicated that our allocation concealment was adequate. Finally, the differences between S-14 and S-10 and between S-10 and T-14 were not statistically significant and our study was not sufficiently powered to directly test these two hypotheses (that the efficacy comparisons are equal between S-14 and S-10 and between S-10 and T-14) because their differences were small. Further studies are also needed to identify the most cost-effective regimen tailored to meet the needs of specific populations,[33] such as those who receive treatment for peptic ulcer disease, those who undergo test-and-treat strategy for non-ulcer dyspepsia, and those who undergo screen-and-treat strategy for gastric cancer.

Our findings lend support to the use of sequential treatment as the standard first-line treatment for *H pylori* infection. Our findings also lend support to the idea that the best eradication regimen should be chosen on the basis of the prevalence of antibiotic-resistant *H pylori* in the region.

Contributors

J-ML, J-TL, Y-CL, and M-SW had the idea for the study, with input from all the other listed contributors from all other authors. J-ML, M-SW, and J-TL designed the study and wrote the protocol. J-ML and J-TL contributed equally in this work. J-ML and Y-CL did the statistical analyses. Y-CL designed the decision model and did the sensitivity analyses. All authors recruited patients to the study. U-CY set up the website to assemble the data from different medical centres. C-TS contributed to the histological assessment. J-ML, Y-CL, and M-SW drafted the paper and all authors commented on drafts and approved the final version.

Conflicts of interests

We declare that we have no conflicts of interest.

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