

Comparison of the effect of clarithromycin triple therapy with or without *N*-acetylcysteine in the eradication of *Helicobacter pylori*: a randomized controlled trial

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Abstract

Background: Whether adjunctive *N*-acetylcysteine (NAC) may improve the efficacy of triple therapy in the first-line treatment of *Helicobacter pylori* infection remains unknown. Our aim was to compare the efficacy of 14-day triple therapy with or without NAC for the first-line treatment of *H. pylori*.

Material and methods: Between 1 January 2014 and 30 June 2018, 680 patients with *H. pylori* infection naïve to treatment were enrolled in this multicenter, open-label, randomized trial. Patients were randomly assigned to receive triple therapy with NAC [NAC-T14, dexamproprazole 60 mg four times daily (q.d.); amoxicillin 1 g twice daily (b.i.d.), clarithromycin 500 mg b.i.d., NAC 600 mg b.i.d.] for 14 days, or triple therapy alone (T14, dexamproprazole 60 mg q.d.; amoxicillin 1 g b.i.d., clarithromycin 500 mg b.i.d.) for 14 days. Our primary outcome was the eradication rates by intention to treat (ITT). Antibiotic resistance and *CYP2C19* gene polymorphism were determined.

Results: The ITT analysis demonstrated *H. pylori* eradication rates in NAC-T14 and T14 were 81.7% [276/338, 95% confidence interval (CI): 77.5–85.8%] and 84.3% [285/338, 95% CI 80.4–88.2%], respectively. In 646 participants who adhered to their assigned therapy, the eradication rates were 85.7% and 88.0% with NAC-T14 and T14 therapies, respectively. There were no differences in compliance or adverse effects. The eradication rates in subjects with clarithromycin-resistant, amoxicillin-resistant, or either clarithromycin/amoxicillin resistant strains were 45.2%, 57.9%, and 52.2%, respectively, for NAC-T14, and were 66.7%, 76.9%, and 70.0%, respectively, for T14. The efficacy of NAC-T14 and T14 was not affected by *CYP2C19* polymorphism.

Conclusion: Add-on NAC to triple therapy was not superior to triple therapy alone for first-line *H. pylori* eradication [ClinicalTrials.gov identifier: NCT02249546].

Keywords: community setting, dexamproprazole, eradication, *Helicobacter pylori*, *N*-acetylcysteine, triple therapy

Received: 19 January 2020; revised manuscript accepted: 15 April 2020.

Ther Adv Gastroenterol

2020, Vol. 13: 1–12

DOI: 10.1177/
1756284820927306

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Introduction

Helicobacter pylori infection is associated with peptic ulcer disease and gastric cancer,^{1,2} and population-wide eradication seems to be the most direct approach to reduce the enormous consequences of *H. pylori* infection. Implementation of *H. pylori* eradication programs requires an effective, simple, and practical antibiotic regimen. However, the efficacy of standard triple therapy containing clarithromycin, amoxicillin, and a proton-pump inhibitor (PPI) for *H. pylori* eradication has fallen below 80% due to the rising prevalence of clarithromycin resistance.^{3,4} Several strategies have been developed to increase the efficacy of first-line *H. pylori* eradication therapies.^{3,4} Previous studies showed that *H. pylori* produce biofilm, an extracellular polymeric matrix [polysaccharides, deoxyribonucleic acid (DNA), proteins, and lipids] with water channels as a strategy to overcome environmental stress and protect itself.^{5,6} Bacteria in biofilms can be more resistant to antibiotics and human defenses than free-living ones by retarding antibiotic diffusion, allowing expression of gene resistance, presence of antibiotic-hydrolyzing enzymes, and decreasing the bacterial growth ratio.^{7,8} Yonezawa *et al.* showed that *H. pylori* biofilm formation decreases the susceptibility to clarithromycin, and that *H. pylori* clarithromycin-resistant mutations are more frequently generated in biofilms than in planktonic cells.⁹ *N*-acetylcysteine (NAC) has anti-biofilm activity against pathogens and was used in treating device-related infection and chronic respiratory-traction diseases.^{10–12} Therefore, NAC is a promising agent to increase the efficacy of *H. pylori* eradication therapy.¹³ Cammarota *et al.* reported that NAC was able to avoid biofilm formation and to destabilize the already-formed biofilm at concentrations of over 10 mg/ml.¹⁴ Some studies have shown NAC has an additive effect on *H. pylori* eradication in first-line therapy,^{15–19} and an Italian study suggested that NAC pretreatment before a culture-guided antibiotic regimen is effective for overcoming *H. pylori* antibiotic resistance in patients with refractory *H. pylori* infection.¹⁴ However, most studies were small sized and hospital based. The antibiotic therapy used in some studies were dual therapy,^{15,19} which were not the recommended regimens according to current guidelines.^{20,21} Whether NAC provides adjunctive effect on standard first-line *H. pylori* treatment, including in the community populations, remains unknown. Besides, antimicrobial susceptibility was not available in previous studies

and the additive effect of NAC to overcome antibiotic resistance still needs further study.

Therefore, we conducted this randomized controlled trial to assess whether adjunctive NAC may increase the efficacy of triple therapy given for 14 days in the first-line *H. pylori* eradication by recruiting both community-based and hospital-based populations.

Materials and methods

Trial design and settings

This multicenter, open-label, randomized trial was conducted in eight participating hospitals in Taiwan and a community population residing in an offshore island (Matsu island). The institutional review board of each participating unit approved the study. Study research staff recruited potential participants and explained the purpose and eligibility requirements of the study to them. Written informed consent was obtained from each subject before enrollment [Clinical Trial.gov identifier: NCT02249546].

Patient enrollment

Symptomatic and asymptomatic adult subjects were invited to participate in our *H. pylori* screening program. Symptomatic patients who underwent esophagogastroduodenoscopy (EGD) for dyspepsia or other symptoms were eligible for enrollment if they had at least positive tests among histology, rapid urease test, culture, and serology. Asymptomatic subjects with a single positive ¹³C-urea breath test (¹³C-UBT) were also eligible for enrollment. Subjects were excluded from the study if any one of the following criteria was present: (a) history of *H. pylori* eradication; (b) history of gastrectomy; (c) gastric malignancy, including adenocarcinoma and lymphoma; (d) contraindication or previous allergic reaction to study drugs (amoxicillin, clarithromycin, NAC, or dexlansoprazole); (e) pregnancy or lactating women; (f) severe concurrent disease. Written informed consent was obtained from all patients before enrollment.

Intervention and assessment of the adverse effect

Eligible patients were randomized to receive either (a) NAC adjunctive triple therapy (NAC-T14) for

14 days: dexlansoprazole delayed release (DR; Dexilant™, Takeda Pharmaceuticals, Osaka, Japan) 60 mg once daily for 14 days, amoxicillin (amoxicillin capsules, China Chemical & Pharmaceutical Co. Ltd., Taipei, Taiwan) 1 g, clarithromycin (Klaricid™ XL, Abbvie SRL, Campoverde, Italy) 500 mg, NAC (Actein, Synmosa Biopharma Co., Taipei, Taiwan) 600 mg (all given twice daily) for 14 days; or (b) triple therapy alone (T14): dexlansoprazole DR 60 mg (once daily) for 14 days, amoxicillin 1 g, clarithromycin 500 mg (all given twice daily) for 14 days. The permuted block randomization sequence with a block size of 4 in a 1:1 ratio was generated by a computer. The sequence was concealed in an opaque envelope and kept by an independent research assistant in the National Taiwan University Hospital until intervention was assigned. All investigators were blind to the randomization sequence. After obtaining written informed consent from eligible subjects, the study nurses contacted the independent assistant at the National Taiwan University Hospital to obtain the next allocation number by phone to ensure adequate allocation concealment.

Outcomes

The primary end-point of the study was the eradication rate. The secondary end-points were the frequency of adverse effects and compliance. All subjects were informed of the common side effects of the studied drugs before eradication therapy and were asked to record these symptoms during treatment. In the hospital setting, we arranged a standardized outpatient clinic interview at the end of treatment. The research staff assessed the adverse events and compliance using a pre-defined case report form and counted the pills not taken by the subjects. In the community setting, the adverse events and compliance were assessed by phone calls. Patients with low compliance as defined by taking less than 80% of the pills and those lost to follow up were excluded from the per protocol (PP) analysis.

Determination of eradication status, phenotypic and genotypic resistance, CYP2C19 polymorphism

H. pylori eradication was defined as a negative ¹³C-UBT at least 6 weeks after completion of treatment. The breath samples were analyzed by an infrared isotope analyzer (IRIS®, Wagner Analysen

Technik GmbH, Bremen, Germany). A delta-over-baseline ≥ 4.0 indicated positive ¹³C-UBT.

For participants who underwent EGD, gastric specimens were obtained. The specimens were cultured and maintained on CDC anaerobe agar (BD, Tokyo, Japan) containing 5% sheep blood under microaerophilic conditions (85% N₂, 10% CO₂, and 5% O₂) at 37°C. Strains were identified if they were Gram negative, positive for urease, oxidase, and catalase, and had spiral or curved rods in morphology. The agar dilution method, with Mueller-Hinton agar (Remel Laboratories, Lenexa, KS), containing aged sheep blood, and incubation at 35°C for 72 h in a microaerophilic, was performed to determine the minimum inhibitory concentrations (MICs) according to the Clinical Laboratory Standards Institute protocols. The breakpoints for amoxicillin, clarithromycin, levofloxacin, tetracycline, and metronidazole resistance were defined as greater than ≥ 0.5 , ≥ 1 , > 1 , > 0.5 , and ≥ 8 mg/l, respectively.^{22,23} The genotypes of *gyrA* and *23S rRNA* were determined by polymerase chain reaction (PCR) followed by direct sequencing using the automatic sequencer (ABI PRISM 3100 Genetic Analyzer; Applied Biosystems, Foster city, California)^{4,23} and *CYP2C19* polymorphism was genotyped by PCR, followed by the restriction fragment-length polymorphism method, as described previously.²⁴

Sample size estimation and statistical analysis

We estimated the eradication of standard triple therapy for 14 days to be 82%.²² Previous trials have reported that the eradication rate in the NAC-containing group was 8–12% higher than that in the control group.^{16–18} The presented trial was designed as a superiority study. To show a 9% difference in eradication rate between the triple-therapy group and NAC-triple-therapy group, a sample size of at least 340 subjects in each group was required to provide a statistical power of 90% at a 5% significance level on a two-sided test, assuming an 8% loss to follow-up rate. Participants who took at least one dose of study medication were recruited in intent-to-treat (ITT) analyses. All protocol violators, such as subjects who failed to take at least 80% of their treatment drugs, or who had unknown post-treatment *H. pylori* status, were excluded from the PP analysis. Subjects who did not return for a follow-up ¹³C-UBT were considered treatment failures.

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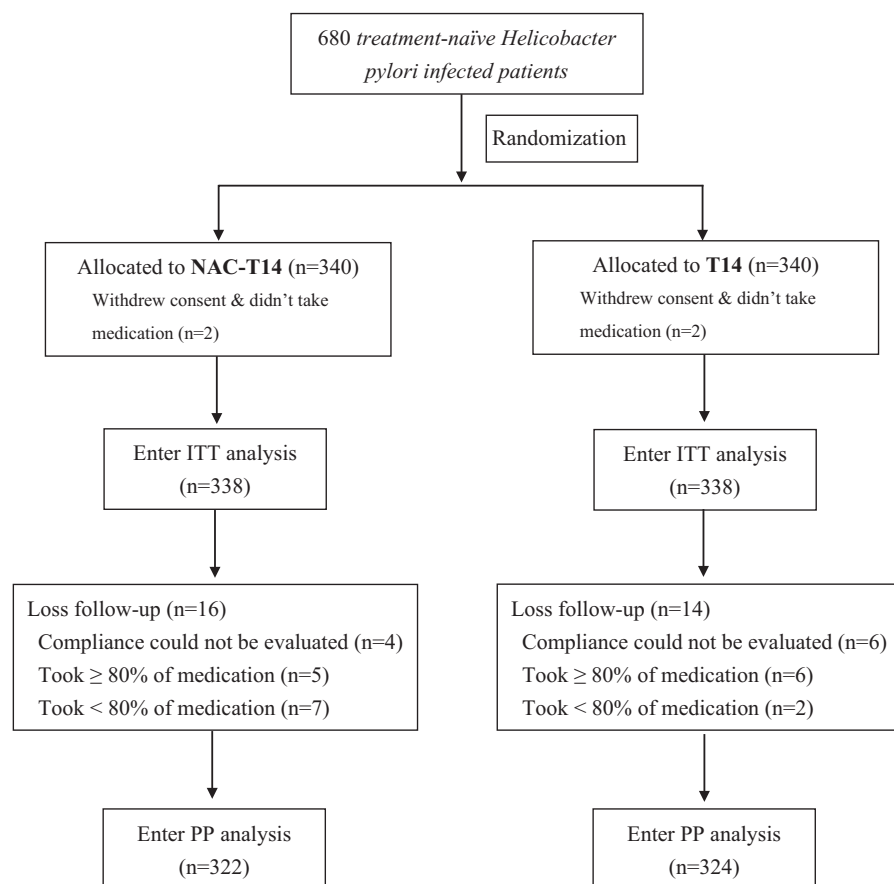


Figure 1. Study flow diagram.

ITT, intention to treat; NAC-T14, *N*-acetylcysteine with adjunctive triple therapy for 14 days; PP, per protocol; T14, triple therapy alone for 14 days.

For statistical analysis, categorical data were compared using the chi-square test or Fisher's exact test, as appropriate. Continuous data were compared using the Student's *t*-test and expressed as mean (standard deviation, SD). All *p*-values were two tailed, with the level of statistical significance specified as 0.05. The 95% confidence interval (95% CI) of the eradication rate of each regimen was calculated. Multiple logistic regression analyses were performed to detect the affecting factors of *H. pylori* eradication in both groups. The odds ratio (OR) and 95% CI were applied to measure the degree of its association. The covariates adjusted in the model included factors with an important biological impact on *H. pylori* treatment, such as age, sex, tobacco smoking (yes or no), peptic ulcer (yes or no), compliance (good compliance was defined as taking at least 80% of the drugs), *CYP2C19* polymorphism (poor metabolizer, PM, or not); *H. pylori* susceptibility or resistance to clarithromycin or amoxicillin as

defined above. The statistical analyses were performed using STATA version 14.0 (StataCorp LP, College Station, TX, USA).

Results

Baseline data and characteristics

We enrolled 680 *H. pylori*-infected patients from 1 January 2014 to 30 June 2018. A detailed study flowchart as shown in Figure 1. There were 453 patients undergoing endoscopy, and gastric biopsy was undertaken for *H. pylori* culture. We performed genotyping of *23S rRNA* in gastric biopsy specimens and it was successfully done in 332 patients. *H. pylori* were isolated in 353 patients, and antibiotic susceptibility data were available in 352 patients.

The baseline demographic characteristics were balanced between the two groups (Table 1). The

antimicrobial susceptibility for clarithromycin, levofloxacin, metronidazole, tetracycline, and amoxicillin was similar across two groups.

Helicobacter pylori eradication rates and adverse events

The eradication rates of NAC-T14 and T14 were 81.7% (276/338, 95% CI 77.5–85.8%) and 84.3% (285/338, 95% CI 80.4–88.2%) in the ITT analysis, respectively ($p=0.36$). The PP analysis showed eradication rates in NAC-T14 group and T14 group were 85.7% (276/322, 95% CI 81.9–89.6%) and 88.0% (285/324, 95% CI 84.4–91.5%), respectively ($p=0.40$). The adjunctive NAC therapy was not superior to standard triple therapy in both ITT and PP analyses (Table 2). The eradication rates in clarithromycin-resistant, amoxicillin-resistant or either clarithromycin/amoxicillin resistant strains were 45.2%, 57.9%, and 52.2% respectively, for NAC-T14, and were 66.7%, 76.9%, and 70.0%, respectively, for T14 (Table 2). For patients with antimicrobial resistance against clarithromycin and/or amoxicillin, adding NAC did not improve the eradication rates.

The frequency of any adverse effects was similar between both groups, except for a higher proportion of bloating in T14 than in NAC-T14 (9.3% versus 4.6%, $p=0.02$; Table 3). There were marginally fewer taste-distortion (12.0% versus 17.2%, $p=0.07$) and constipation (2.9% versus 5.9%, $p=0.08$) events in patients treated with NAC-T14 than those treated with T14. The compliance (taking at least 80% of the drugs) was similar in patients treated with NAC-T14 or T14 (95.8% versus 96.2%, $p=0.79$). Less than 5% of patients discontinued the drugs due to adverse effects in both groups.

Comparison between hospital and community settings

The subgroup analysis results are shown in Table 4. In the community population, the prevalence of peptic ulcer disease and antibiotic resistance were lower. Of the reported adverse events, the compliance to treatment in the hospital population was similar to that in the community population, while subjects in the community setting had slightly higher treatment discontinuation rate and loss-to-follow-up rate. The treatment efficacies (ITTs) for *H. pylori* infection were 82.8% (82/99)

Table 1. Demographic characteristics and prevalence of antibiotic resistance.

	NAC-T14 (n = 338)	T14 (n = 338)	p
Male	49.7% (168/338)	51.8% (175/338)	0.59
Age, mean (SD)	50.5 (13.3)	51.0 (13.3)	0.64
Cigarette smoking	22.8% (77/338)	17.8% (60/338)	0.10
Alcohol drinking	34.0% (115/338)	30.5% (103/338)	0.32
Peptic ulcer	51.5% (136/264)	46.3% (117/253)	0.23
CYP2C19-PM	15.7% (36/229)	13.8% (31/225)	0.72
BMI	24.6 (4.02)	24.5 (3.90)	0.68
Obesity	8.6% (29/338)	7.4% (25/338)	0.57
Antimicrobial resistance			
23S rRNA mutation (tissue)	13.5% (23/171)	11.3% (18/159)	0.56
23S rRNA mutation (strain)	16.7% (27/162)	15.2% (23/151)	0.73
Clarithromycin resistance	18.2% (33/181)	18.9% (32/169)	0.87
Metronidazole resistance	20.0% (36/180)	22.9% (39/170)	0.50
Amoxicillin resistance	10.6% (19/180)	7.7% (13/170)	0.35
Levofloxacin resistance	18.8% (34/181)	20.6% (35/170)	0.67
Tetracycline resistance	0% (0/180)	0% (0/168)	
<i>H. pylori</i> test positive, n (%)			
Serology	99.5% (210/211)	96.6% (197/204)	0.03
Rapid urease test	93.2% (164/176)	93.9% (168/179)	0.80
Histology	91.5% (172/188)	94.7% (179/189)	0.22
Culture	86.8% (184/212)	86.4% (171/198)	0.89
Urea breath test	99.3% (149/150)	100% (158/158)	0.30
BMI, body mass index; F, female; M, male; NAC-T14, N-acetylcysteine adjunctive triple therapy for 14 days; PM, poor metabolizer; SD, standard deviation; T14, triple therapy for 14 days.			

and 81.9% (86/105) for NAC-T14 and T14, respectively, in the community population, and the difference was not significant.

Table 2. Eradication rates.

	NAC-T14		T14		<i>p</i>
	% (n/N)	95% CI	% (n/N)	95% CI	
Overall analysis					
ITT analysis	81.7% (276/338)	77.5–85.8%	84.3% (285/338)	80.4–88.2%	0.36
PP analysis % (n/N)	85.7% (276/322)	81.9–89.6%	88.0% (285/324)	84.4–91.5%	0.40
Eradication rates by antimicrobial susceptibility					
CLA-R	45.2% (14/31)	27.3–64.0%	66.7% (20/30)	47.2–82.7%	0.09
AMX-R	57.9% (11/19)	15.7–84.3%	76.9% (10/13)	50.4–100.0%	0.57
CLA-R or AMX-R	52.2% (24/46)	37.2–67.2%	70.0% (28/40)	55.2–84.8%	0.09
CLA-R and AMX-R	25% (1/4)	–54.5% to 100%	66.7% (2/3)	–76.8% to 100%	0.35
AMX-R, amoxicillin resistant; CI, confidence interval; CLA-R, clarithromycin resistant; ITT, intention to treat; N, total study population number; NAC-T14, N-acetylcysteine adjunctive triple therapy for 14 days; PP, per-protocol; T14, triple therapy for 14 days.					

Table 3. Adverse events of eradication therapy.

	NAC-T14	T14	<i>p</i>
Any adverse effects	37.3% (115/308)	42.8% (124/290)	0.18
Dizziness	5.5% (17/308)	5.9% (17/290)	0.86
Skin rash	3.6% (11/308)	2.4% (7/290)	0.41
Headache	4.2% (13/308)	3.1% (9/290)	0.47
Taste distortion	12.0% (37/308)	17.2% (50/290)	0.07
Abdominal pain	6.5% (20/308)	9.7% (28/290)	0.16
Nausea	3.3% (10/308)	2.4% (7/290)	0.54
Diarrhea	16.9% (52/308)	18.3% (53/290)	0.66
Constipation	2.9% (9/308)	5.9% (17/290)	0.08
Bloating	4.6% (14/308)	9.3% (17/290)	0.02
Vomiting	0.7% (2/308)	1.7% (5/290)	0.22
Discontinued drugs due to adverse effects	2.9% (9/308)	3.8% (11/290)	0.55
Took less than 80% of drugs	4.2% (13/308)	3.8% (11/290)	0.79
Took the drugs correctly	95.8% (294/308)	96.6% (281/290)	0.62
NAC-T14, N-acetylcysteine adjunctive triple therapy for 14 days; T14, triple therapy for 14 days.			

Table 4. Characteristics and outcomes according to settings.

	Hospitals			Communities		<i>p</i>
	NAC-T14	T14		NAC-T14	T14	
Male	45.6% (109/239)	47.2% (110/233)	0.73	59.6% (59/99)	61.9% (65/105)	0.74
Age, mean (SD)	51.1 (12.7)	51.6 (12.3)	0.67	49.1 (14.6)	49.6 (15.2)	0.80
Cigarette smoking	21.3% (51/239)	18.0% (42/233)	0.37	26.3% (26/99)	17.1% (18/105)	
Alcohol drinking	30.1% (72/239)	27.0% (63/233)	0.46	43.4% (43/99)	38.1% (40/105)	0.11
Peptic ulcer	39.8% (128/216)	39.3% (111/214)	0.12	16.7% (8/48)	15.4% (6/39)	0.87
<i>CYP2C19</i> -PM	15.7% (36/229)	13.8% (31/225)	0.50	NA	NA	
BMI	24.5 (4.0)	24.3 (3.6)	0.57	25.0 (4.0)	25.0 (4.5)	0.98
Obesity	7.1% (17/239)	5.6% (13/233)	0.50	12.1 (12/99)	11.4 (12/105)	0.88
Resistance						
<i>23S rRNA</i> mutation (tissue)	14.2% (18/127)	12.3% (15/122)	0.66	11.4% (5/44)	8.1% (3/37)	0.63
<i>23S rRNA</i> mutation (strain)	17.4% (23/132)	17.2% (22/128)	0.96	13.3% (4/30)	4.4% (1/23)	0.27
Clarithromycin resistance	19.2% (29/151)	20.3% (30/148)	0.82	13.3% (4/30)	9.1% (2/22)	0.64
Metronidazole resistance	22.5% (34/151)	23.1% (34/148)	0.90	6.9% (2/29)	21.8% (5/23)	0.12
Amoxicillin resistance	12.7% (19/150)	8.8% (13/147)	0.28	0% (0/30)	0% (0/23)	
Levofloxacin resistance	21.2% (32/151)	23.8% (35/147)	0.59	6.7% (2/30)	0.0% (0/23)	0.21
Tetracycline resistance	0% (0/151)	0% (0/146)		0% (0/29)	0% (0/22)	
Eradication rate						
ITT	81.2% (194/239)	85.4% (199/233)	0.22	82.8% (82/99)	81.9% (86/105)	0.86
PP	84.7% (194/229)	89.2% (199/223)	0.15	88.2% (82/93)	85.2% (86/101)	0.54
Any adverse events	39.5% (94/238)	43.5% (100/230)	0.38	28.6% (20/70)	36.7% (22/60)	0.33
Discontinued drugs due to adverse effects	2.5% (6/238)	3.0% (7/230)	0.73	4.3% (3/70)	6.7% (4/60)	0.55
Took at least 80% drugs	96.2% (229/238)	96.5% (222/230)	0.86	94.3% (66/70)	95.0% (57/60)	0.86
Took the drugs correctly	95.0% (226/238)	97.0% (223/230)	0.27	98.6% (69/70)	95.0% (57/60)	0.24
Lost follow up	4.2% (10/239)	4.3% (10/233)	0.95	6.1% (6/99)	3.8% (4/105)	0.46
BMI, body mass index; NAC-T14, <i>N</i> -acetylcysteine adjunctive triple therapy for 14 days; T14, triple therapy for 14 days.						

Factors affecting eradication rates

Tables 4 and 5 summarize the factors affecting *H. pylori* eradication rates. The eradication rates were significantly affected by clarithromycin or amoxicillin resistance in both NAC-T14 and T-14 (Tables 2 and 5). The results were consistent,

using genotyping or MIC to detect clarithromycin resistance. These findings suggested that the presence of antimicrobial resistances significantly affected the eradication rates for both groups, regardless of NAC adjunctive treatment. Multiple regression analyses showed that poor compliance

Table 5. Factors affecting *H. pylori* eradication rate.

	NAC-T14	T14
Univariate analyses		
Clarithromycin resistance (MIC)		
Resistant	45.2% (14/31)**	66.7% (20/30)**
Susceptible	91.7% (132/144)**	96.3% (131/136)**
23s rRNA mutation (tissue)		
Yes	36.4% (8/22)**	75% (12/16)*
No	92.4% (133/144)**	92.8% (128/138)*
23s rRNA mutation (tissue)		
Yes	34.6% (9/26)**	66.7% (14/21)**
No	92.4% (121/131)**	94.4% (119/126)**
Amoxicillin resistance (MIC)		
Resistant	57.9% (11/19)*	76.9% (10/13)
susceptible	86.5% (134/155)*	92.2% (141/153)
Clarithromycin or amoxicillin resistance (MIC)		
Resistant	52.2% (24/46)**	70% (28/40)**
Susceptible	94.5% (121/128)**	97.6% (122/125)**
Compliance (took at least 80% drugs)		
Yes	85.9% (249/290)	89.7% (245/273)
No	83.3% (5/6)	77.8% (7/9)
Peptic ulcer		
Present	86.5% (115/133)	91.3% (105/115)
Absent	81.3% (100/123)	90.7% (117/129)
Smoking		
Yes	79.2% (57/72)	84.2% (48/57)
No	87.6% (219/250)	88.8% (237/267)
CYP2C19 polymorphism		
PM	91.8% (31/34)	93.6% (29/31)
IM/EM	82.9% (55/187)	89.2% (165/185)
Settings		
Hospital	84.7% (194/229)	89.2% (199/223)
Community	88.2% (82/93)	85.2% (86/101)
* $p < 0.05$. ** $p < 0.001$. EM, extensive metabolizer; IM, intermediate metabolizer; NAC-T14, N-acetylcysteine adjunctive triple therapy for 14 days; MIC, minimum inhibitory concentration; PM, poor metabolizer; T14, triple therapy for 14 days.		

and antimicrobial resistance against clarithromycin were risk factors associated with NAC-T14 and T14 treatment failure (Table 6).

Discussion

The presented study showed adjunctive treatment with NAC did not have an additive effect on the *H. pylori* eradication rate with first-line triple therapy. Our result confirmed findings of Emami *et al.*²⁵ but was against most of the earlier studies.^{15–19} However, Zala *et al.*¹⁹ and Gurbuz *et al.*¹⁵ evaluated the effect of NAC on the eradication of *H. pylori* in patients receiving dual therapy, which was not recommended by current guidelines. Yoon *et al.*,¹⁸ Karbasi *et al.*,¹⁶ and Hamidian *et al.*¹⁷ suggested that the addition of NAC to a two- or three-antibiotic regimen yielded numerically higher *H. pylori*-eradicating rate, compared with therapies without NAC. These studies were underpowered and did not reach statistical significance. Our study is the largest trial investigating the role of adjunctive treatment with NAC in standard first-line triple therapy. It provided evidence to demonstrate that NAC containing 14-day triple therapy was not superior to standard 14-day triple therapy, and the result was also supported by the recent meta-analysis.²⁶

Several reports have shown that antibiotic resistance might be the most important factor affecting the success of *H. pylori* eradication.^{4,27} One of the hypotheses that NAC improves *H. pylori* efficacy is to destroy bacterial biofilm and overcoming the problem of antibiotic resistance.²⁸ Nevertheless, most of the previous trials did not provide information on antibiotic resistance. The main novelty of our study included the collection of antibiotic susceptibility data from the study populations and it provides a chance to investigate whether NAC overcomes the antimicrobial resistance. Our analyses disclosed that the eradication rate for patients with clarithromycin- or amoxicillin-resistant strains in NAC-T14 was not superior to that in T14 groups (for clarithromycin: 45.2% versus 66.7%, respectively, $p = 0.09$; for amoxicillin: 57.9% versus 76.9%, $p = 0.26$; Table 2) and the finding was consistent when the different methods to detect clarithromycin resistance were employed (Table 5). The multiple regression analysis also demonstrated clarithromycin resistance was the most influential factor in the determination of treatment efficacy in both NAC-T14

and T14 groups, which suggesting NAC may not overcome antimicrobial resistance (Table 6).

Another novel finding provided by our study is the use of Dexilant™ (dexlansoprazole DR) in *H. pylori* treatment. Dexlansoprazole, an R-enantiomer of lansoprazole, is a PPI with three to five times greater maximum concentration, and a longer elimination half-life than S-lansoprazole.²⁹ Dexilant™ employs a dual delayed-release technology designed for potent and long-lasting acid suppression. A study concluded that once-daily 60 mg dexlansoprazole was equivalent to 15.5 mg twice-daily omeprazole in Western populations, determined by the ability to maintain the median pH at 6 or higher for 24h.³⁰ Our study, which used once-daily Dexilant™, demonstrated similar *H. pylori* eradication rates, compared with the studies employing widely-used twice-daily lansoprazole triple therapy (ITT: 82.4% *versus* 82.3–85.7%, PP: 86.2% *versus* 87.1–91%),^{4,22,31} and the efficacy was not significantly affected by *CYP2C19* polymorphism (Tables 5, 6). In addition to potent acid suppression of dexlansoprazole, the improved effectiveness of dexlansoprazole-based anti-*H. pylori* therapy may reflect more corpus gastritis and smaller parietal-cell masses in Asian populations. Our study suggests anti-*H. pylori* therapy with once-daily Dexilant™ would be a more convenient option for Asians in the real world.

The strength of this study included its large sample size, the analysis of eradication rate according to antibiotic susceptibility, the evaluation of host *CYP2C19* polymorphism, and the consideration of different enrollment settings. Treatment of asymptomatic carriers in primary care settings may make them more vulnerable to the side effects, which are highly associated with the length and complexity of the antimicrobial regimen.^{32,33} Few reports addressed the efficacy of antibiotic treatment for *H. pylori* infection in primary care settings. Our findings support our previous trial and a study from investigators in Latin America that 14-day triple therapy is an acceptable empiric therapy with good treatment adherence for *H. pylori* infection in community populations.^{4,34}

Our study had several limitations. First, the *H. pylori* eradication rate between the two study groups was quite similar in both ITT and PP analyses (NAC-T14 *versus* T14: ITT, 81.7%

Table 6. Multivariate analyses for factors affecting eradication rates*.

	NAC-T14		T14	
	Odds ratio (95% CI)	<i>p</i>	Odds ratio (95% CI)	<i>p</i>
Clarithromycin resistance	19.6 (5.7–66.6)	<0.001	15.0 (3.7–61.1)	<0.001
Amoxicillin resistance	7.7 (1.9–34.0)	0.004	3.5 (0.6–21.1)	0.18
Poor compliance	59.9 (2.6–1385.6)	0.02	17.4 (0.8–359.8)	0.07
Peptic ulcer	0.3 (0.09–1.0)	0.06	0.7 (0.9–3.0)	0.46
Smoking	1.3 (0.8–2.1)	0.31	1.7 (0.9–3.0)	0.10
<i>CYP2C19</i> PM	0.7 (0.1–4.0)	0.69	0.2 (0.02–2.0)	0.17

*ORs in the multiple logistic regression models adjusted for clarithromycin resistance, amoxicillin resistance, compliance, peptic ulcer, *CYP2C19* polymorphism, age, and sex.
CI, confidence interval; NAC-T14, N-acetylcysteine adjunctive triple therapy for 14 days; OR, odds ratio; PM, poor metabolizer; T14, triple therapy for 14 days.

versus 84.3%; PP, 85.7% *versus* 88%). However, it would be very difficult to recruit more than 2000 subjects in both arms to see such a narrowed difference. Our study is still the largest trial investigating the role of NAC in *H. pylori* eradication and we believe our data have contributed to *H. pylori* studies. Second, not all the participants had an endoscopy for *H. pylori* culture, and the results of antibiotic susceptibility tests were only available in approximately 51% of patients in the present study. This percentage was mainly related to the enrollment settings, and the technical difficulty of culture and MIC testing. However, this limitation did not affect the main purpose of our study (NAC-T14 *versus* T14). The treatment efficacies were indeed similar between the groups with and without antibiotic resistance data and selection bias was unlikely. Third, the reason why NAC adjunctive therapy did not show the promising efficacy in treating antibiotic-resistant *H. pylori* as shown in the previous study is unknown. One possible explanation is that Cammarota *et al.*¹⁴ gave NAC pretreatment for 1 week before salvage antibiotic therapy for refractory *H. pylori* infection, while antibiotics were given concurrently with NAC in our study. However, routine

application of pretreatment with NAC in the first-line *H. pylori* treatment would raise the concern of poor compliance due to its complexity.

In conclusion, empirical dexamprazole DR-based triple therapy for 14 days is well tolerated and effective for *H. pylori* eradication, compared with triple therapy using twice-daily PPI. Adding a bio-film destabilizing agent, NAC, in dexamprazole DR-based triple therapy is not superior to the standard dexamprazole DR-based triple therapy in treating *H. pylori*, and is not necessary for the first-line treatment of *H. pylori* infection.

Acknowledgments

The authors would like to express their special thanks to the staff of the Eighth Core Lab, Department of Medical Research, National Taiwan University Hospital, and the first-line healthcare workers in Matsu Island.

Yi-Chia Lee, Jyh-Ming Liou and Ming-Shiang Wu contributed equally as co-corresponding authors of this study.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Author contributions

The study was conceived by C-C Chen with input from J-M Liou, Y-C Lee and M-S Wu and all the other listed contributors from the Taiwan Gastrointestinal Disease and Helicobacter Consortium. The Taiwan Gastrointestinal Disease and Helicobacter Consortium investigators were: the 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); others investigators of the Taiwan Helicobacter Consortium in this study: Tsu-Yao Cheng (Taipei), 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), Ching-Tai Lee (Kaohsiung), Wen-Lun Wang (Kaohsiung), and Wen-Shiung Chang (Taipei).

C-C Chen and JC Luo designed the study and wrote the protocol. C-C Chen, J-C Luo, J-Y Lee, T-H Yang, C-C Yu, C-C Kuo, M-C Chiu, M-J Bair, P-Y Chen, C-K Chou, C-Y Chen, C-Y Chang, Y-C Hsu, C-H Tseng, W-F Hsu, W-H Hu, M-H Tsai, C-L Hsieh, M-J Chen, Y-C Lee and M-S Wu recruited patients to the study. C-C Chen prepared the statistical analyses. C-C Chen drafted the article, which was critically revised by three senior authors, J-M Liou, Y-C Lee, and M-S Wu. M-S Wu supervised the hospital setting and Y-C Lee and T-Y Liu supervised the community setting in Matsu Island. 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.

Funding

The study was funded by the National Taiwan University Hospital (grant no. 108-T11), the National Taiwan University Hospital and Taipei Veterans General Hospital (Grant Number: VN104-10, VN105-07), the Takeda Company (grant no. IISR-2014-100737), the Ministry of Science and Technology, Executive Yuan, ROC, Taiwan (grant no. TCTC-TR2 106-2321-B-002-025, and MOST 107-3017-F-002-002), the Ministry of Health and Welfare of Taiwan (grant no. 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 in Taiwan (grant no. NTU-107L9014-1), and the Liver Disease Prevention & Treatment Research Foundation, Taiwan. 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.

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