1 The efficacy of a 14-day modified quadruple therapy containing
2 amoxicillin, tetracycline and high dose metronidazole and proton-pump
3 inhibitors as an empirical third line *helicobacter pylori* eradication
4 treatment in Taiwan

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Abstract

The antibiotics resistances to amoxicillin, tetracycline was low in Taiwan even after multiple *H. pylori* treatment failures and high dose metronidazole could overcome antibiotics resistance. In real world practice, susceptibility-guided treatments are not widely available. Therefore, we assessed the efficacy of 14-day modified quadruple therapy containing amoxicillin, tetracycline and high dose metronidazole and PPI as an empirical third-line rescue *H. pylori* treatment. This study was conducted by analyzing 70 consecutive prospectively registered patients who failed two times *H. pylori* eradication. All of them received endoscopy for *H. pylori* culture. Seven patients were lost to follow up. They were then treated according to the antibiotic susceptibility testing reports (Cultured group, n=39). Those who failed *H. pylori* culture were prescribed with a modified 14-day quadruple therapy containing esomeprazole 40 mg twice daily, amoxicillin 1 g twice daily, tetracycline 500 mg four times daily and metronidazole 500 mg three times daily (empirical group, n=24). Follow-up urea breath test was performed 8 weeks later. The eradication rates attained by Cultured group and empirical group were 89.7% (95% confidence interval [CI] = 72.72%-97.11%) and 58.3% (95% CI=36.61%-77.86%), in the per protocol analysis (p=0.004); 81.4%(95% CI=66.60%-91.61%) and 51.8% (95% CI=31.9%-71.29%), in the intention-to-treat analysis (P=0.014). Culture-guided therapy was the clinical factors influencing the efficacy of *H. pylori* eradication (OR: 0.16; 95% CI: 0.04-0.60, p=0.006). In conclusion, empirical 14-day modified

54 quadruple therapy is not acceptable as an alternative third-line rescue *H. pylori* treatment

55 probably but the success rate of the third-line susceptibility-guided treatment was only

56 moderate (<90%).

57 **Keywords:** Third line *Helicobacter pylori* eradication; empirical quadruple therapy;

58 culture-guided therapy.

Introduction

Helicobacter pylori (*H. pylori*) infection is one of the most common bacterial infections in the world and was classified as grade I carcinogen (1-3). It could slowly induce chronic gastritis, which progresses through the premalignant stages of atrophic gastritis, intestinal metaplasia, and dysplasia, and then finally to gastric cancer (4, 5). Meanwhile, successful eradication of *H. pylori* has greatly reduced the recurrence of peptic ulcers (6). The worldwide standard triple therapy consisted with one proton pump inhibitor (PPI) and amoxicillin and clarithromycin, and in place of amoxicillin for who are allergic to it, metronidazole has been one alternative. However, *H. pylori* may develop resistance to the prescribed antibacterial like clarithromycin as standard first line therapy and may acquire resistance by acquisition and recombination of genes from other bacteria (7). The resistance battle against *H. pylori* continue, many people still abuse self-medication of antibiotic like levofloxacin, which helps *H. pylori* to develop more drugs resistances (8-10).

After failure of second-line treatment, culture with susceptibility testing or molecular determination of genotype resistance is recommended by the Maastricht V/Florence-Consensus Report (11). The secondary resistances to clarithromycin and levofloxacin are high in patients who failed regimens containing these antibiotics (12-13). Therefore, the reuse of clarithromycin and levofloxacin empirically should be avoided in the third line regimens. On the other hand, there is a major limitation to this therapeutic line

because of low sensitivity of culture-based advice. Moreover, majority of hospitals and clinics have no facility for culture with antibiotics susceptibility testing or molecular test of genotype resistance. The more effective third line regimens for *H. pylori* should be warranted in the future. The issue on the urge for an ideal empirical third line *H. pylori* rescue therapy has been raised. The Toronto consensus recommended the choice of antibiotics empirically according to medication history (14). However, earlier studies have generally shown poor eradication rates with Rifabutin based (15) or Rifaximin based (16) as third line rescue therapy of only 63-66% regardless of combination with amoxicillin, levofloxacin or clarithromycin.

In Taiwan, *H. pylori* culture with susceptibility testing or molecular determination of genotype resistance is recommended in third line rescue therapy in adherence to Taiwan Consensus Report (17). However, we may encounter patients who failed *H. pylori* culture or refuse to do further endoscopy and molecular determination of genotype resistance is not available. Liou et al reported that secondary antibiotic resistances to amoxicillin and tetracycline are low in Taiwanese patients who failed at least two eradications (18). The addition of a PPI helped overcome metronidazole resistance and double dose twice a day is recommended (19). In our previous studies, we observed that high dose metronidazole may overcome metronidazole (20). Therefore, the present study aims to evaluate the efficacy of this simple modified empirical 14-day quadruple therapy with esomeprazole, amoxicillin,

metronidazole and tetracycline as the third line regimens for *H. pylori* eradication.

Results

Patients' demographic and baseline characteristics

Figure 1 summarizes the patient disposition. Seven patients were lost to follow up. Therefore, 63 patients were included in the per protocol analysis. Their baseline characteristics are shown in table 1. The mean age is 58.9 ± 10.1 years. 36.5% are male patients and 63.5% are female patients. There was no significant difference between the culture group and empirical group in age, gender, smoking and alcohol habits, peptic ulcer history, and the endoscopic finding

Eradication rate and susceptibility of *H. Pylori* culture

Figure 2 demonstrated the antibiotics resistances after two *H. pylori* treatment failures. Clarithromycin resistance strains were found in 79.5% (31/39) of the patients. Levofloxacin-resistant strains were 94.9% (37/39). Metronidazole resistance strains were 66.7% (26/39). Amoxicillin resistance was 2.6% (1/39). No strains developed resistance to tetracycline. Table 2 demonstrates the major outcomes of eradication rate. The eradication rates attained by Cultured group and empirical group were 89.7% (95% confidence interval [CI] = 72.72%-97.11%) and 58.3% (95% CI=36.61%-77.86%), respectively in the per protocol

analysis ($p=0.004$) 81.4% (95% CI=66.6%-91.61%) and 51.8% (95% CI=31.9%-71.29%), respectively, in the intention-to-treat analysis ($P=0.014$). Despite the high metronidazole resistance after two treatment failures, the eradication rate in the patients with was as high as 84% in this patient cohort (Table 3).

Adverse events and compliance

The adverse event occurred in 17 patients (22.9%). There was no significant difference between two groups in adverse events (25.6% vs. 29.2%, $p=0.758$) (Table 2). The greatest side effect from the use of medications was the digestive system such as the presence of nausea, abdominal pain and constipation (Table 4). All patients completed their course of treatment, therefore, with 100% of compliance.

Factors influenced the efficacy of anti-*H. pylori* therapy

Table 5 shows the univariate analysis of all demographic data and factors. In univariate analysis, there was only one clinical factor, culture vs. empirical therapy, with p -value 0.004. Multivariate analysis, culture vs. empirical therapy was the only clinical factor influencing *H. pylori* eradication (OR: 0.16, 95% CI: 0.04-0.60, $p=0.006$) (Table 6).

Discussion

According to Maastricht V/Florence Consensus Report (11), after failure of second line

135 therapy for eradication of *H. Pylori*, clinically, the best choice of therapy should be based on
136 the cultivation of *helicobacter pylori* and get its susceptibility to provide a better solution for
137 the population. However, determination of the MIC for *H. pylori* is not widely available,
138 because it is time consuming, inconvenient and relatively expensive. Besides, the successful
139 culture rate ranges from 75% to 90% (21, 22). Genotypic resistance-guided test is a quick and
140 effective assay to decide the choice of antibiotics sensitive to *H. Pylori*. Liou et al (18)
141 reported a study in the North Taiwan about genotypic resistance-guided test in third-line
142 treatment, improving the overall eradication rate in patients who received clarithromycin-,
143 levofloxacin- and tetracycline-based sequential therapies were 78.9% (15/19), 92.2%
144 (47/51) and 71.4% (25/35) in strains susceptible to clarithromycin, levofloxacin and
145 tetracycline, respectively. The eradication rate will get the optimal result in the patients
146 who are taking the levofloxacin-based regiment if the genotype was sensitive to
147 levofloxacin. However, in current study, the patient failing *H. pylori* eradication after using
148 the 1st line standard triple clarithromycin-based therapy and 2nd line levofloxacin-based
149 therapy developed the high resistant rate in clarithromycin 79.5% (31/39) and levofloxacin
150 94.9% (37/39). Conversely, the amoxicillin and tetracycline resistance was as low as 2.6%
151 (1/39), and 0%. Therefore, we used a simple and modified 14-day high-dose quadruple
152 therapy with esomeprazole, amoxicillin, metronidazole and tetracycline as the empirical third
153 line regiment for *H. pylori* eradication for patients who failed *H. pylori* culture and refused

further endoscopy procedure. There were several reasons for the empirical use of this modified quadruple empirical therapy. First, the prevalence of tetracycline and amoxicillin resistance, even in patients who have failed multiple treatments, remains low worldwide (17, 23). Our results confirmed that the prevalence of tetracycline resistance was 0% in patients failing two eradication treatments and developed 0~10% in five years' sequential change in our previous culture report (20). Second, the impact of tetracycline and metronidazole resistance on the eradication rate remains controversial (21, 24-27). It is also because of high dose metronidazole was effective for eradication therapy, despite a relatively high resistance rate. Third, several randomized controlled trials (28, 29) compared two different durations of eradication therapy, and demonstrated that the longer duration is more effective. Therefore, we set the third line treatment course as 14 days.

In this study, per-protocol analysis, culture guided therapy achieved significantly higher eradication rate than empirical therapy (89.7% vs. 58.3%, $p=0.004$). This study suggests that this empirical quadruple therapy is not acceptable in third line *H. pylori* eradication therapy. However, there was no definite factor found in detail analysis to predict the successful rate, except the culture-guided vs. empirical group (p -value is 0.004). Chen et al reported a > 90 % eradication rate by using a bismuth-containing quadruple therapy with metronidazole and amoxicillin is an alternative to classical bismuth quadruple therapy for *H. pylori* third line rescue treatment (30). At this point, we wonder why reinvent the wheel as a similar regime

with different doses and doing intervals ha been reported to be successful. The possible explanation could be probably related to a drug–drug interaction between amoxicillin and tetracycline which exhibit low in vitro antibiotic resistance (31). The success in Chen’s study could be due to the synergism that occurs between metronidazole and its hydroxymetabolite and between either the analog or amoxicillin / tetracycline, both of which may contribute to the efficacy of both the amoxicillin–metronidazole and tetracycline–metronidazole combinations (32). It was proposed that bacteriostatic drugs like tetracycline might interfere with the bactericidal action of penicillin (33). The bactericidal action is to inhibit cell wall formation which is dependent on how fast the bacteria are multiplying. Tetracycline may reduce the effectiveness of penicillin because it is a bacteriostatic antibiotics and can inhibit the cellular protein synthesis that is required for cell division. Eventually, susceptibility-guided treatment is still recommended for third line *H. pylori* eradication therapy as Maastricht V/Florence Consensus Report (11).

It was not so surprised that the eradication rate in the patients with metronidazole resistance was as high as 84% (21/25) as had been observed in many previous publications [20, 29, 34]. The possible explanation was environment changes in the oxygen pressure in the stomach, as metronidazole-resistant *H. pylori* isolates can become susceptible to metronidazole under low oxygen conditions in vitro (27, 35). This phenomenon of high dose metronidazole overcoming antibiotics resistance was also documented in our previous study

(29, 33). In Spain, Ignasi Puig et al made a systematic review and meta-analysis on therapy based on culture-guided susceptibility (36), they found that the eradication rate of *H. pylori* was only with an average of 72%. In our study, our eradication rate was also < 90%.

In real world practice, despite the introduction of culture testing or molecular genotype resistance after failure of second-line treatment, it is not routinely available in majority of medical institutions. In this regional study, we noted that if the patients failed the clarithromycin-based standard triple therapy and levofloxacin-based therapy, the resistant strains to clarithromycin and levofloxacin were very high. Initial hypothesis was that the empirical modified 14-day high-dose quadruple therapy with amoxicillin, tetracycline, high dose metronidazole and esomeprazole, could be an alternative the third line regiment for *H. pylori* eradication. Despite that the good drug compliance and was easier to meet patients' adherence, the eradication success rate did not fulfill our expectation probably due to the drug-drug interaction between amoxicillin and tetracycline.

There were some limitations in this study. First, the study sample size was small. Second, we were unable to turn this study into a randomized trial because we felt it was not ethical to randomize patients with multiple *H. pylori* eradication failures with available culture susceptibility results. In conclusion, empirical 14-day modified quadruple therapy is not acceptable as an alternative third-line rescue *H. pylori* treatment in spite of the low amoxicillin and tetracycline resistances probably due to the drug-drug interaction between

amoxicillin and tetracycline but the success rate of the third-line susceptibility-guided treatment was only moderate (<90%). The optimal management of rescue treatments requires further research.

Materials and methods

Patients

A total of 70 prospective registered consecutive *H. pylori* infected patients, who failed *H. pylori* eradication by using the 1st line standard triple therapy and 2nd line levofloxacin-based therapy between 1, January 2015 and 31, December 2017 at Kaohsiung Chang Gung Memorial Hospital, Taiwan, were recruited in this study. Based on the international guidelines, these patients had received endoscopy for *H. pylori* culture and consequent antibiotic susceptibility testing. Seven patients were lost to follow up. All patients would be at least 18 years of age and had received endoscope examinations that showed peptic ulcers or gastritis. The confirmation of *H. pylori* eradication failure was defined as patients with either one positive 13 C-UBT or any two positive of the rapid urease test, histology and culture after second-line eradication therapy. Criteria for exclusion included (a) ingestion of antibiotics, bismuth, or PPIs within 4 weeks before; (b) patients with allergic history to the medications used; (c) patients with previous gastric surgery; (d) pregnant women. They were then treated according to the antibiotic susceptibility testing

reports as 3rd line therapy (Cultured group, n=39). Those who failed *H. pylori* culture and did not want to repeat another endoscopy for *H. pylori* culture were prescribed with a simple empirical 14-day modified quadruple therapy containing esomeprazole 40 mg twice daily, amoxicillin 1 g twice daily, tetracycline 500 mg four times daily and metronidazole 500 mg three times daily (empirical group, n=24). To assess eradication efficacy after the completion of anti-*H. pylori* therapy urea breath test was used to confirm the *H. pylori* status at 8 weeks. The absence of *H. pylori* after eradication therapy was defined as a negative result of urea breath test at 8 weeks (9, 37).

The primary outcome variables are the eradication rate, presence of adverse events, and level of patient compliance. Demographic information including age, gender, social history of smoking, alcohol consumption, previous peptic ulcer history and medical history were collected via electrical medical records. This study was approved by both the Institutional Review Board and Ethics Committee of Chang Gung Memorial Hospital, Taiwan (IRB 201800034B0).

Culture and antimicrobial susceptibility testing

One biopsy specimen from the antrum and one from the corpus were obtained for *H. pylori* isolation using previously described culture methods (10, 11). The biopsy specimens were cultured on plates that contained Brucella chocolate agar with 7% sheep blood and were

incubated for 4-5 d under micro-aerobic conditions. The minimal inhibitory concentration (MIC) was determined by the agar dilution test. The *H. pylori* strains were tested for susceptibility to amoxicillin, Clarithromycin, levofloxacin, metronidazole and tetracycline by using the E-test method (AB BIODISK, Solna, Sweden). *H. pylori* strains had MIC values of ≥ 0.5 , ≥ 1 , ≥ 1 , ≥ 4 and ≥ 8 mg/L, which were considered to be the resistance breakpoints for amoxicillin, Clarithromycin, levofloxacin, metronidazole and tetracycline, respectively (38)

Statistical analysis

By using the SPSS program (Statistical Package for the Social Sciences version 23, Chicago, IL, USA), Chi-square tests with or without Yates' correction for continuity and Fisher's exact tests were used when appropriate to compare the major outcomes between groups. Eradication rates were analyzed by per-protocol (PP) approaches. The PP analysis excluded patients with unknown *H. pylori* status following therapy and those with major protocol violations. A P-value < 0.05 was considered statistically significant. To determine the independent factors that affected treatment response, the clinical parameters were analyzed by univariate and multivariate analysis.

Conflict of interest

The authors declare that there is no conflict of interest with respect to the publication of this

268 document.

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404 **Figure legend**

405 Figure 1. Disposition of patients.

406 Figure 2. The antibiotics resistances after two *H. pylori* treatment failures.

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Resistance Rate

Table 1. Demographic data and endoscopic appearances of the two patient groups

Characteristics	Culture-guided therapy (n =39, %)	Empirical therapy (n =24,%)	P-value
Age (year) (mean $\pm$ SD)	58.7 $\pm$ 10.4	58.3 $\pm$ 10.0	0.869
Gender (male/female)	13/26(33.3/66.7)	10/14(41.7/58.3)	0.512
Smoking	4(10.3)	1(4.2)	0.075
Alcohol consumption	6(15.4)	3(12.5)	0.755
Previous history of peptic ulcer	18(46.2)	13(54.2)	0.293
Endoscopic Findings			
Gastritis	15(38.5)	12(50.0)	0.063
Gastric ulcer	20(51.3)	5(20.8)	
Duodenal ulcer	1(2.6)	6(25.0)	
Gastric and duodenal ulcer	3(7.7)	1(4.2)	

Table2. Major outcomes of eradication therapy

	Eradication rate		
	Culture-guidedgroup	Empirical group	P-value
Intention To Treat	81.4% (35/43)	51.8% (14/27)	0.014
Per-protocol	89.7% (35/39)	58.3% (14/24)	0.004
Adverse event	25.6% (10/39)	29.2% (7/24)	0.758
Compliance	100 % (39 /39)	100% (24 /24)	-

Table 3. Antibiotic resistance and *H. pylori* eradication rate

Effect of amoxicillin, tetracycline and metronidazole resistance on <i>Helicobacter pylori</i> eradication rates in the PP population		
Culture	Total	Eradication rate, N(%)
AMO-S TET-S MET-S	13	13 (100)
AMO-R TET -S MET-S	0	-
AMO-S TET -R MET-S	0	-
AMO-S TET -S MET-R	25	21(84)
AMO-R TET -R MET-S	0	-
AMO-R TET -S MET-R	1	1 (100)
AMO-S TET -R MET-R	0	-
AMO-R TET -R MET-R	0	-
Total	39	35(89.7)
AMO: amoxicillin; TET: tetracycline; MET, metronidazole; S: sensitive, R, resistant. PP: per protocol		

Table 4. Adverse events of patients

Adverse events	Culture-guided therapy (n =39, %)	Empirical therapy (n =24, %)	P-value
Abdominal pain	3 (7.7)	2 (8.3)	0.528
Nausea/vomiting	3 (7.7)	2 (8.3)	0.528
Constipation	2 (5.1)	1 (4.2)	0.862
Dizziness	1 (2.6)	1 (4.2)	0.725
Headache	1 (2.6)	1 (4.2)	0.725
Skin rash	0 (0)	0 (0)	-
Diarrhea	0 (0)	0(0)	-

Table 5. Univariate analysis of the clinical factors influencing the efficacy of *H. pylori* eradication

Clinical factors		Eradicated		Not eradicated			P-value
		No	%	No	%	Total	
Sex	Male	18	78.3	5	21.7	23	0.944
	Female	31	77.5	9	22.5	40	
Age	<60 years	25	75.8	8	24.2	33	0.686
	≥60 years	24	80.0	6	20.0	30	
Smoking	No	44	75.9	14	24.1	58	0.460
	Yes	5	100.0	0	0	5	
Alcohol consumption	No	40	74.1	14	25.9	54	0.083
	Yes	9	100.0	0	0	9	
Previous history of peptic ulcer	No	25	75.8	8	24.2	33	0.387
	Yes	24	80.0	6	20.0	30	
Hypertension	No	35	76.1	11	23.9	46	0.595
	Yes	14	82.4	3	17.6	17	
Diabetes Mellitus	No	40	76.9	12	23.1	52	0.723
	Yes	9	81.8	2	18.2	11	
Chronic renal failure	No	49	79.0	13	21.0	62	0.059
	Yes	0	0	1	100.0	1	
Cardiac vascular disease	No	46	78.0	13	22.0	59	0.890
	Yes	3	75.0	1	25.0	4	
Chronic hepatitis	No	44	78.6	12	21.4	56	0.668
	Yes	5	71.4	2	28.6	7	
Psychological problem	No	47	77.0	14	23.0	61	0.442
	Yes	2	100.0	0	0	2	
Hyperthyroidism	No	48	77.4	14	22.6	62	0.590
	Yes	1	100.0	0	0	1	
Culture vs, Empirical	Empirical	14	58.3	10	41.7	24	0.004
	Culture-guided	35	89.7	4	10.3	39	
Compliance	Complete treatment	49	77.8	14	22.2	63	-
	Not complete	0	0	0	0	0	

Table 6. Multivariate analysis of the clinical factors influencing the efficacy of *H. pylori* eradication

Clinical factor	Odds ratio (95% CI)	<i>P</i> -value
Culture guided		
Vs. Empirical therapy	0.16 (0.04~0.60)	0.006