Trend of changes in antibiotic resistance in *Helicobacter pylori* from 2013 to 2019: a multicentre report from Taiwan

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Abstract

Background: Antibiotic resistance plays a crucial role in the treatment failure of *Helicobacter pylori (H. pylori)* infection. This study aimed to determine the trend of changes in the primary, secondary and tertiary antibiotic resistance of *H. pylori* in Taiwan over the last 7 years.

Methods: We retrospectively analysed *H. pylori*-infected isolates from patients with primary resistance ($n = 1369$), secondary resistance ($n = 196$) and tertiary resistance ($n = 184$) from January 2013 to December 2019. The *H. pylori* strains were tested for susceptibility to amoxicillin, clarithromycin, levofloxacin, metronidazole and tetracycline using the Epsilometer test method.

Results: A progressively higher primary resistance rate was observed for clarithromycin (11.8–20.4%, $p = 0.039$ in χ^2 test for linear trend), levofloxacin (17.3–38.8%, $p < 0.001$) and metronidazole (25.6–42.3%, $p < 0.001$) among naïve patients who received first-line eradication therapy. The dual primary resistance to clarithromycin and metronidazole also progressively increased in a linear trend (2.4–10.4%, $p = 0.009$). For secondary resistance, an increase was observed for levofloxacin (30.5–64.7%, $p = 0.006$) and metronidazole (40.5–77.4%, $p < 0.001$). For tertiary resistance, the observed increase was even more significant for levofloxacin (65.9–100.0%, $p = 0.106$) and metronidazole (44.4–88.2%, $p < 0.001$). The resistance to amoxicillin and tetracycline remained very low in Taiwan regardless of primary, secondary and tertiary resistance.

Conclusion: Primary, secondary and tertiary antibiotic resistance to clarithromycin, levofloxacin and metronidazole for *H. pylori* has been increasing in Taiwan since 2013. Treatment should be targeted for eradication success rates of more than 90%. Third-line treatment should be based on antibiotic susceptibility.

Keywords: antibiotic resistance, *Helicobacter pylori*, seven-year trend, Taiwan

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Introduction

Helicobacter pylori (*H. pylori*) is a microaerophilic gram-negative flagellate bacterium that may lead to diseases, such as duodenal/gastric ulcer disease, and the development of a gastric neoplasm. Given this relationship with human diseases, eradication of *H. pylori* in individuals may be the best course of action.[1] The prevalence of antibiotic resistance of *H. pylori* varies among countries and may be partly determined by geographical factors.[2] Knowledge of the prevalence of antibiotic resistance of *H. pylori* in different geographic areas is important because treatment for *H. pylori* infection is often based on empirical data. If the infecting strain is resistant to antibiotics, therapy is highly likely to fail.

Standard triple therapy, which consists of a proton pump inhibitor (PPI), amoxicillin, and clarithromycin or metronidazole, was developed in the 1990s and has been recommended as the first-line eradication therapy due to a favourable eradication rate at that time. In recent years, mounting evidence has indicated a progressively

declining eradication rate of standard triple therapy due to the increase in primary clarithromycin-resistant strains.[3,4] Fortunately, several treatments with various antibiotic combinations that can increase eradication rates to more than 90% emerged slightly more than a decade ago. These treatment options include high-dose dual therapy, hybrid and reverse hybrid therapies, and non-bismuth quadruple therapies. For second-line rescue therapy, levofloxacin-containing triple or quadruple therapy, preferably a 14-day course, is recommended in most consensus reports.[5,6] However, the eradication rates dropped to unacceptable levels when levofloxacin-resistant strains existed.[7,8] Clearly, antibiotic resistance is one of the most important factors that determines the success of eradication. However, antibiotic resistance has increased in Taiwan and other countries over time.[9,10] The clinical decision for optimal eradication regimens should be supported by referring to the relative efficacies stratified by antibiotic resistance and safety profiles.[11] Therefore, continuously monitoring the regional resistance rates was mandatory. In this multicentre study, we collected the data of *H. pylori*-infected isolates from the past 7 years to determine the trends in the primary, secondary and tertiary antibiotic resistance of *H. pylori* in Taiwan.

Materials and methods

Ethics statement

This is a multicentre study conducted at Kaohsiung Chang Gung Memorial Hospital, Kaohsiung Medical University and Hospital, and Kaohsiung Veterans General Hospital (Kaohsiung, Taiwan). This protocol was approved by the institutional review board and the Ethics Committee of Chang Gung Memorial Hospital (IRB-202001199B0).

The Ethics Committee waived the requirement for informed consent for this retrospective study and all the data were analysed anonymously. None of our patients were minors or children.

Patients

We analysed *H. pylori*-infected isolates from patients before first-line eradication therapy ($n = 1369$), second-line eradication therapy ($n = 196$) and third-line eradication therapy ($n = 184$) between January 2013 and December 2019. All isolates from patients who had been previously treated for *H. pylori* infection or who had been exposed to any antibiotics according to our hospital's chart recording system were excluded. Exclusion criteria for this study included: (a) ingestion of antibiotics, bismuth or PPIs within the prior 4 weeks, (b) previous gastric surgery, (c) coexistence of a serious concomitant illness (for example, decompensated liver cirrhosis, uraemia) and (d) pregnancy.

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.[12,13] All stock cultures were maintained at –80°C in Brucella broth (Difco Laboratories, Detroit, MI, USA) supplemented with 20% glycerol (Sigma Chemical Co., St. Louis, MO, USA). 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 Epsilometer 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, tetracycline and metronidazole, respectively, according to the European Committee on Antimicrobial Susceptibility Testing.[14]

Statistical analysis

A χ^2 test for linear trends was used to assess the trends of antibiotic resistance over time from 2013 to 2018. A p value < 0.05 was considered statistically significant.

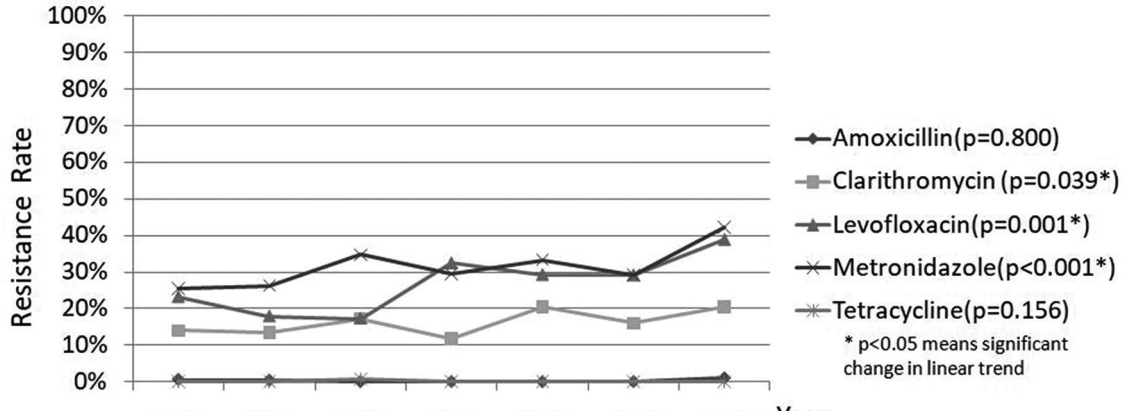
Results

Table 1 shows the characteristics of the patients included in each group. In Figure 1, a progressively higher primary resistance rate was observed for clarithromycin (11.8–20.4%, $p = 0.039$ in the χ^2 test for linear trend), levofloxacin (17.3–38.8%, $p < 0.001$) and metronidazole (25.6–42.3%, $p < 0.001$) among patients who received first-line eradication therapy. The primary combined resistance to clarithromycin and metronidazole also progressively increased in a linear trend from 2.4% to 10.4% ($p = 0.009$).

Table 1. Clinical characteristics of patients.

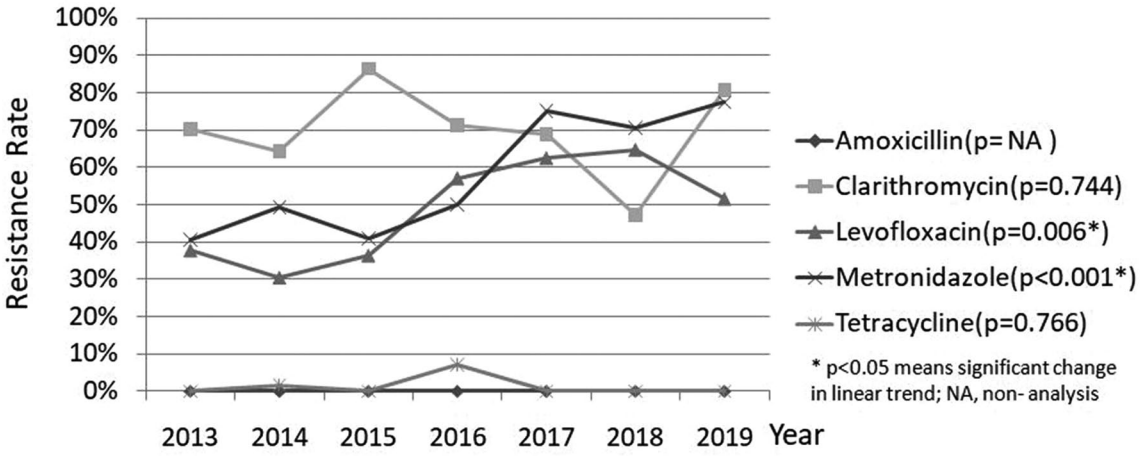
Characteristics	First line *n* = 1369 *n* (%)	Second line *n* = 196 *n* (%)	Third line *n* = 184 *n* (%)	*p*-value
Age, years, mean ± SD	54.0 ± 11.9	55.6 ± 11.2	55.3 ± 10.7	0.077
18–29	31 (2.3)	5 (2.6)	4 (2.2)	
30–39	135 (9.9)	12 (6.1)	8 (4.3)	
40–49	306 (22.4)	29 (14.8)	35 (19.0)	0.009
50–59	420 (30.7)	72 (36.7)	67 (36.4)	
60–69	360 (26.3)	61 (31.1)	62 (33.7)	
⩾70	117 (8.5)	17 (8.7)	8 (4.3)	
Male	679 (49.6)	75 (38.3)	73 (39.7)	0.001
Female	690 (50.4)	121 (61.7)	111 (60.3)	
Diabetes	130 (9.5)	19 (9.7)	14 (7.6)	0.698
Hypertension	256 (18.7)	50 (25.5)	34 (18.5)	0.074
Smoking	240 (17.5)	20 (10.2)	28 (15.2)	0.031
Drinking	101 (7.4)	12 (6.1)	14 (7.6)	0.802

SD, standard deviation.



% Resistance \ year	2013 (n=164)	2014 (n=251)	2015 (n=202)	2016 (n=136)	2017 (n=240)	2018 (n=175)	2019 (n=201)	*p*-value
Amoxicillin	0.6	0.4	0	0	0	0	1.0	0.800
Clarithromycin	14.0	13.5	17.3	11.8	20.4	16.0	20.4	0.039
Levofloxacin	23.2	17.9	17.3	32.4	29.2	29.1	38.8	0.001
Metronidazole	25.6	26.3	34.7	29.4	33.3	29.1	42.3	<0.001
Tetracycline	0	0	0.8	0	0	0	0	0.156
Dual resistance to Clarithromycin and metronidazole	2.4	5.2	8.4	5.1	8.3	5.7	10.4	0.009

Figure 1. The trend of annual antibiotic resistance rates in the first-line treatment of *Helicobacter pylori* (*n* culture = 1369).



Year / % Resistance	2013 (n=37)	2014 (n=59)	2015 (n=22)	2016 (n=14)	2017 (n=16)	2018 (n=17)	2019 (n=31)	*p*-value
Amoxicillin	0	0	0	0	0	0	0	NA
Clarithromycin	70.3	64.4	86.4	71.4	68.8	47.1	80.6	0.744
Levofloxacin	37.8	30.5	36.4	57.1	62.5	64.7	51.6	0.006
Metronidazole	40.5	49.2	40.9	50.0	75.0	70.6	77.4	<0.001
Tetracycline	0	1.7	0	7.1	0	0	0	0.766

Figure 2. The trend of annual antibiotic resistance rates in the second-line treatment of *Helicobacter pylori* (*n* culture = 196).

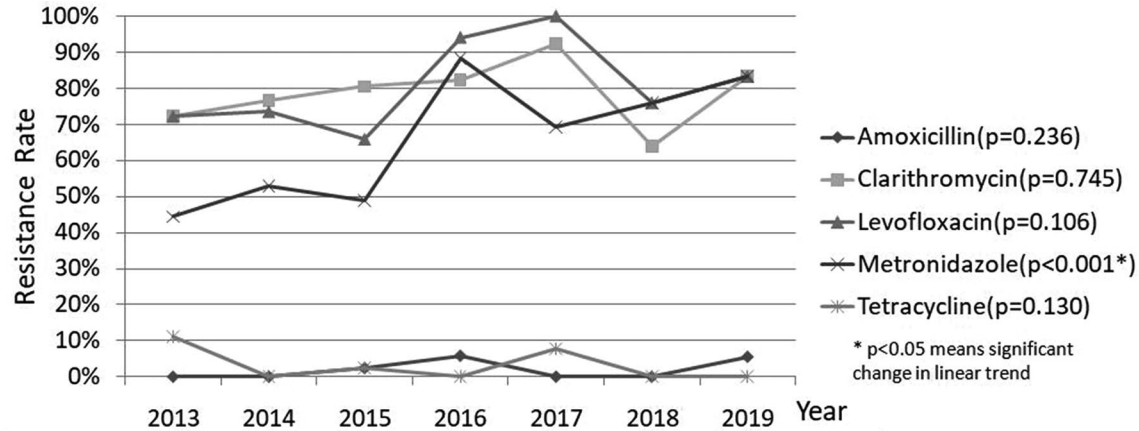
Similar upward resistance trends were also found in secondary resistance to levofloxacin (30.5–64.7%, $p = 0.006$) and metronidazole (40.5–77.4%, $p < 0.001$). The resistance rate for clarithromycin increased but was not statistically significant in secondary resistance (47.1–86.4%, $p = 0.744$) (Figure 2). A similar upward resistance trend was found for the tertiary resistance to levofloxacin (65.9–100.0%, $p = 0.106$), and a significantly upward resistance to metronidazole was found (44.4–88.2%, $p < 0.001$). Similar to the secondary resistance of clarithromycin, the tertiary resistance rate was not significantly increased (64.0–83.3%, $p = 0.745$) (Figure 3).

The primary, secondary and tertiary resistance of amoxicillin and tetracycline remained very low in Taiwan (amoxicillin: 0.4–1.0% for primary resistance, $p = 0.800$; 0% for secondary resistance; and 0–5.9% for tertiary resistance, $p = 0.236$; tetracycline: 0–0.8% for primary resistance, $p = 0.178$; 0–7.1% for secondary resistance, $p = 0.766$; and 0–11.1% for tertiary resistance, $p = 0.130$).

Discussion

Antibiotic resistance is a crucial factor for the failure of *H. pylori* eradication. Resistance is associated with the individual and a combination of genetic point mutations. For clarithromycin resistance, point mutations have been found in the rrl gene encoding two 23S rRNA nucleotides (*2142* and *2143*) or associated with the efflux pump system.[15,16] Metronidazole resistance in *H. pylori* is relevant to the mutational inactivation of the *redox*-related gene (*frxA*, *rdxA*).[17] Quinolone resistance is determined in *H. pylori* (*N87* and *D91*) in the quinolone resistance-determining region of the *gyrA* gene of *H. pylori*.[8] Amoxicillin resistance is associated with alterations in penicillin-binding proteins.[10] The triple base pair 16S rDNA mutation of AGA (926–928) to TTC mediates high-level tetracycline resistance in *H. pylori*.[18,19]

The current study observed that there was a progressively increasing primary resistance rate for clarithromycin, levofloxacin and metronidazole and dual resistance to clarithromycin and metronidazole among naïve patients who received first-line



Year / % Resistance	2013 (n=18)	2014 (n=34)	2015 (n=41)	2016 (n=17)	2017 (n=13)	2018 (n=25)	2019 (n=36)	*p*-value
Amoxicillin	0	0	2.4	5.9	0	0	5.6	0.236
Clarithromycin	72.2	76.5	80.5	82.4	92.3	64.0	83.3	0.745
Levofloxacin	72.2	73.5	65.9	94.1	100.0	76.0	83.3	0.106
Metronidazole	44.4	52.9	48.8	88.2	69.2	76.0	83.3	<0.001
Tetracycline	11.1	0	2.4	0	7.7	0	0	0.130

Figure 3. The trend of annual antibiotic resistance rates in the third-line treatment of *Helicobacter pylori* (*n* culture = 184).

eradication therapy. For both secondary and tertiary resistance, the increases in the resistance to levofloxacin and metronidazole were even more significant. The primary, secondary and tertiary resistances for amoxicillin and tetracycline remained very low in Taiwan. In this study, one biopsy specimen from the antrum and one from the corpus were obtained for *H. pylori* isolation using previously described culture methods. Pichon *et al.* described the difference between gastric distribution, density and diversity of *H. pylori* in the stomach antrum and corpus, as these regions could include different levels of resistance.[20] This study observed that no specific area in the stomach could be highlighted with the absence of *H. pylori* detection, suggesting that a single biopsy could be enough for *H. pylori* detection.

The prevalence of resistance rates appears to be partly determined by geographical factors.[9] Savoldi *et al.* performed a systematic review to assess the distribution of *H. pylori* resistance to commonly used antibiotics and to measure the association between antibiotic resistance and treatment failure over 10 years. Resistance to clarithromycin, metronidazole and levofloxacin was found to cross the threshold of 15% in most World Health Organization regions.[9] The prevalence of primary clarithromycin resistance was 16–20% in Europe, 23–44% in the Eastern Mediterranean Region and 30–38% in the Western Pacific Region. A resistance rate of 10% was recorded in the Americas Region (4–16%) and the South-East Asia Region (5–16%). Primary dual resistance to clarithromycin and metronidazole was 19% in the Mediterranean Region and <10% in the other regions. In the current study, a progressively higher resistance rate was observed for clarithromycin (11.8–20.4%, $p = 0.039$) and metronidazole (25.6–42.3%, $p < 0.001$) among patients who received first-line eradication therapy. These findings indicated that southern Taiwan had developed a high clarithromycin resistance of >20% over the last 7 years. Importantly, the primary dual resistance to clarithromycin and metronidazole also significantly increased in a linear trend from 2.4% to 10.4% ($p = 0.009$). The significant increase in resistance to clarithromycin found in this study for the last 7 years was probably due to the large-scale prescription of this antibiotic in Taiwan. For instance, in real-world clinical practice, standard clarithromycin-based triple therapy has been widely used to treat naïve *H. pylori*-infected patients. However, this standard triple therapy has been abandoned as a first-line treatment option for *H. pylori* infection in most countries.[21–23]

Alternatively, bismuth or non-bismuth-containing quadruple therapy, high-dose PPI dual therapy, and hybrid therapy can attain >90% success as first-line *H. pylori* eradication regimens in both areas with high (>15%) and low (<15%) clarithromycin resistance.[5,6,22,24–26]

Recent studies reported the emergence of a novel potassium-competitive acid blocker (vonoprazan) that provides a stronger and longer-lasting effect on gastric acid suppression than other PPIs.[27] Vonoprazan-containing dual therapy (vonoprazan 20 mg + amoxicillin 750 mg twice per day) achieved a higher eradication rate than vonoprazan-containing triple therapy against clarithromycin-resistant strains (92.3% *versus* 76.2%; $p = 0.048$).[28] This was reasonable as the resistance to amoxicillin and tetracycline was negligible (<5%) in most of the countries, and vonoprazan produces stronger and longer-lasting effects on the gastric acid suppression.[9]

Fluoroquinolones, especially levofloxacin, are used in the eradication of *H. pylori* as one of the components in second-line rescue therapy for *H. pylori* eradication. Unfortunately, lengthy quinolone exposure can easily develop bacterial resistance and remains a problematic issue that influences the efficacy of eradication. The current study observed upward resistance trends for secondary resistance to levofloxacin from 30.5% to 64.7% ($p = 0.006$). Although the secondary resistance to levofloxacin increased to above 50%, the Maastricht V/Florence Consensus Report still recommends fluoroquinolone–amoxicillin triple or quadruple therapy as a second-line therapy for *H. pylori* infection.[6] Realistically, a systemic review and meta-analysis reported by Chen *et al.* revealed that levofloxacin-amoxicillin triple therapy achieved an overall eradication rate of 78% after failure of first-line non-bismuth quadruple therapy and concomitant therapies.[27] The key message from these studies was that the success rates of levofloxacin–amoxicillin triple or quadruple therapy are poor in the presence of fluoroquinolone resistance.[8,29]

The secondary resistance rate for clarithromycin increased in the current study, although it did not reach a statistically significant power. It is generally not recommended to use clarithromycin as rescue therapy in real-world practice. The secondary resistance to metronidazole ranged from 40.5% to 77.4% ($p < 0.001$). Obviously, it is necessary to continue searching for other options with high eradication rates. Chuah and Liang *et al.* reported that '5-plus 5' days of levofloxacin and metronidazole-containing sequential therapy achieved a >90% eradication rate as a second-line H. pylori therapy compared with the 80.5% eradication rate in levofloxacin-containing triple therapy.[30] It is possible that the addition of high-dose metronidazole can overcome metronidazole resistance and enhance the effectiveness of levofloxacin-containing therapy. The other advantage was that this sequential therapy shortened the levofloxacin treatment to only 5 days.

The low resistance rates to both amoxicillin and tetracycline imply that they are good candidates for second-line therapies for *H. pylori* eradication. Amoxicillin resistance is associated with alterations in penicillin-binding proteins, and multiple site mutations (*PBP1*, *PBP2* and *PBP3*) are required to cause amoxicillin resistance.[31] Moreover, amoxicillin is a time-dependent antibiotic and therefore requires prolonged time to attain a higher plasma concentration than the minimal inhibitory concentration to achieve higher plasma levels of the drug to achieve the optimal bactericidal effect on an intragastric pH of 5.5 or higher. Yang *et al.* reported that high-dose dual therapy (PPI and amoxicillin) may be a promising second-line therapy and has an eradication efficacy of 89%.[32] A meta-analysis of randomised controlled trials showed that 14-day bismuth quadruple therapy was a good choice for rescue treatment after failure of clarithromycin-containing triple therapy.[33,34] Interestingly, Hsu *et al.* reported that a better grade A eradication rate was achieved using a 10-day tetracycline–levofloxacin quadruple therapy comprising a PPI, bismuth, tetracycline and levofloxacin than a 10-day levofloxacin–amoxicillin triple therapy (98% *versus* 69%) after failure of standard triple therapy, bismuth quadruple therapy or non-bismuth quadruple therapy.[35] In the Shinozaki *et al.* systemic review study, a vonoprazan-based regimen had significant superiority over a PPI-based regimen for second-line *H. pylori* eradication therapy [odds ratio (OR) 1.51, 95% confidence interval (CI) 1.27–1.81, $p < 0.001$]. Vonoprazan-based regimens may play an important role in second-line *H. pylori* eradication therapy in the future.[36]

The upward tertiary resistance trend was more significant in this study (levofloxacin: 65.9–100.0%, $p = 0.106$ and metronidazole: 44.4–88.2%, $p < 0.001$).

The resistance to amoxicillin and tetracycline remained low for tertiary resistance (amoxicillin: 0–5.9%, $p=0.236$; tetracycline: 0–11.1%, $p=0.130$). According to the Maastricht V/Florence and Taiwan Consensus Report,[5,6] after failure of second-line therapy for the eradication of *H. pylori*, the best therapeutic choice should be based on the cultivation of *H. pylori* to determine its susceptibility to medications. Culture-guided therapy was an important clinical factor influencing the efficacy of *H. pylori* eradication (OR 0.16, 95% CI 0.04–0.60; $p=0.006$).[35] Based on the low resistance rate of amoxicillin, high-dose PPI and amoxicillin dual therapy are often recommended as an alternative third-line regimen.[37] Compared with the PPI-based regimen, the vonoprazan-based regimen (vonoprazan 20 mg, amoxicillin 750 mg and sitafloxacin 100 mg, b.i.d.) showed a significant difference in the third-line treatment of *H. pylori* in Japan.[38] Recently, Saito *et al.* confirmed that the success rates of third-line treatment for the eradication of clarithromycin- and sitafloxacin-resistant *H. pylori* were significantly higher for vonoprazan-based triple therapy than for esomeprazole-based triple therapy (91.7% *versus* 20.0%, $p<0.001$).[39] They explained that the success could be due to the strong acid inhibition by vonoprazan and low amoxicillin resistance. However, more evidence is needed to support the actual effect of the vonoprazan-amoxicillin dual combination in third-line *H. pylori* eradication therapy.

The current study has some limitations. First, the clinical *H. pylori* eradication rate was not presented in this study due to various eradication regimens. The secondary and tertiary antibiotic resistance developed diversity according to previous eradication regimens. Second, we were unable to provide any further genetic data for these resistant strains and point mutations in *H. pylori* DNA. In reality, these techniques are not feasible to apply in the current practice. However, the non-invasive Amplidiag *H. pylori* ClariR assay developed by Mobidiag (Espoo, Finland) for the detection of *H. pylori* and clarithromycin resistance with stool samples could be promising in the future.[20]

In conclusion, primary, secondary and tertiary antibiotic resistance to clarithromycin, levofloxacin and metronidazole in *H. pylori* has been increasing in Taiwan since 2013. Treatment should be targeted for eradication success rates of more than 90%. Third-line treatment should be based on antibiotic susceptibility.

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Author contributions

Chuah SK and Chen KY designed the research, critically revised the manuscript for important intellectual content and approved the paper.

Tai WC, Hsu PI, Wu DC, Kuo CH, Tsai FW, Lee CL performed the research.

Liang CM analysed the data and wrote the paper.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

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References

1. Wu W, Yang Y and Sun G. Recent insights into antibiotic resistance in *Helicobacter pylori* eradication. *Gastroenterol Res Pract* 2012; 2012: 723183.
2. De Francesco V, Giorgio F, Hassan C, *et al.* Worldwide *H. pylori* antibiotic resistance: a systematic review. *J Gastrointestin Liver Dis* 2010; 19: 409–414.
3. Megraud F, Coenen S, Versporten A, *et al.* *Helicobacter pylori* resistance to antibiotics in Europe and its relationship to antibiotic consumption. *Gut* 2013; 62: 34–42.
4. Sasaki H, Nagahara A, Hojo M, *et al.* Ten-year trend of the cumulative *Helicobacter pylori* eradication rate for the 'Japanese eradication strategy'. *Digestion* 2013; 88: 272–278.

5. Sheu BS, Wu MS, Chiu CT, *et al.* Consensus on the clinical management, screening-to-treat, and surveillance of *Helicobacter pylori* infection to improve gastric cancer control on a nationwide scale. *Helicobacter* 2017; 22: e12368.
6. Malfertheiner P, Megraud F, O'Morain CA, *et al.* Management of *Helicobacter pylori* infection-the Maastricht V/Florence consensus report. *Gut* 2017; 66: 6–30.
7. Hsu PI, Tsai FW, Kao SS, *et al.* Ten-day quadruple therapy comprising proton pump inhibitor, bismuth, tetracycline, and levofloxacin is more effective than standard levofloxacin triple therapy in the second-line treatment of *Helicobacter pylori* infection: a randomized controlled trial. *Am J Gastroenterol* 2017; 112: 1374–1381.
8. Tai WC, Lee CH, Chiou SS, *et al.* The clinical and bacteriological factors for optimal levofloxacin-containing triple therapy in second-line *Helicobacter Pylori* eradication. *PLoS One* 2014; 9: e105822.
9. Savoldi A, Carrara E, Graham DY, *et al.* Prevalence of antibiotic resistance in *Helicobacter pylori*: a systematic review and meta-analysis in World Health Organization regions. *Gastroenterology* 2018; 155: 1372–1382. e17.
10. Wu IT, Chuah SK, Lee CH, *et al.* Five-year sequential changes in secondary antibiotic resistance of *Helicobacter pylori* in Taiwan. *World J Gastroenterol* 2015; 21: 10669–10674.
11. Yeo YH, Shiu SI, Ho HJ, *et al.* First-line *Helicobacter pylori* eradication therapies in countries with high and low clarithromycin resistance: a systematic review and network meta-analysis. *Gut* 2018; 67: 20–27.
12. Hsu PI, Hwang IR, Cittelly D, *et al.* Clinical presentation in relation to diversity within the *Helicobacter pylori* cag pathogenicity island. *Am J Gastroenterol* 2002; 97: 2231–2238.
13. Chuah SK, Tai WC, Hsu PI, *et al.* The efficacy of second-line anti-*Helicobacter pylori* therapy using an extended 14-day levofloxacin/amoxicillin/proton-pump inhibitor treatment–a pilot study. *Helicobacter* 2012; 17: 374–381.
14. The European Committee on Antimicrobial Susceptibility Testing – EUCAST, https://www.eucast.org/ast_of_bacteria/previous_versions_of_documents/ (accessed 19 September 2015).
15. Arslan N, Yılmaz Ö and Demiray-Gürbüz E. Importance of antimicrobial susceptibility testing for the management of eradication in *Helicobacter pylori* infection. *World J Gastroenterol* 2017; 23: 2854–2869.
16. Bina JE, Alm RA, Uria-Nickelsen M, *et al.* *Helicobacter pylori* uptake and efflux: basis for intrinsic susceptibility to antibiotics in vitro. *Antimicrob Agents Chemother* 2000; 44: 248–254.
17. Tsugawa H, Suzuki H, Satoh K, *et al.* Two amino acids mutation of ferric uptake regulator determines *Helicobacter pylori* resistance to metronidazole. *Antioxid Redox Signal* 2011; 14: 15–23.
18. Gerrits MM, Berning M, Van Vliet AH, *et al.* Effects of 16S rRNA gene mutations on tetracycline resistance in *Helicobacter pylori*. *Antimicrob Agents Chemother* 2003; 47: 2984–2986.
19. Chopra I and Roberts M. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiol Mol Biol Rev* 2001; 65: 232–260.
20. Pichon M, Tran CT, Motillon G, *et al.* Where to biopsy to detect helicobacter pylori and how many biopsies are needed to detect antibiotic resistance in a human stomach. *J Clin Med* 2020; 9: E2812.
21. Yao CC, Kuo CM, Hsu CN, *et al.* First-line Helicobacter pylori eradication rates are significantly lower in patients with than those without type 2 diabetes mellitus. *Infect Drug Resist* 2019; 12: 1425–1431.
22. Tai WC, Liang CM, Lee CH, *et al.* Seven-day nonbismuth containing quadruple therapy could achieve a grade "A" success rate for first-line Helicobacter pylori eradication. *Biomed Res Int* 2015; 2015: 623732.
23. Kuo YT, Liou JM, El Omar EM, *et al.* Primary antibiotic resistance in Helicobacter pylori in the Asia-Pacific region: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 2017; 2: 707–715.
24. Tai WC, Liang CM, Kuo CM, *et al.* A 14-day esomeprazole- and amoxicillin-containing high dose dual therapy achieves high eradication rate in the first line anti-helicobacter pylori treatment in Taiwan: a prospective randomized trial. *J Antimicrob Chemother* 2019; 74: 1718–1724.
25. Tsay FW, Wu DC, Yu HC, *et al.* Randomized controlled trial shows that both 14-day hybrid and bismuth quadruple therapies cure most patients with Helicobacter pylori infection in populations with moderate antibiotic resistance. *Antimicrob Agents Chemother* 2017; 61: e00140-17.
26. Hsu PI, Tsay FW, Graham DY, *et al.* Equivalent efficacies of reverse hybrid and bismuth

quadruple therapies in eradication of helicobacter pylori infection in a randomized controlled trial. *Clin Gastroenterol Hepatol* 2018; 16: 1427–1433.

27. Sakurai Y, Mori Y, Okamoto H, *et al.* Acid-inhibitory effects of vonoprazan 20 mg compared with esomeprazole 20 mg or rabeprazole 10 mg in healthy adult male subjects–a randomised open-label cross-over study. *Aliment Pharmacol Ther* 2015; 42: 719–730.
28. Suzuki S, Gotoda T, Kusano C, *et al.* Seven-day vonoprazan and low-dose amoxicillin dual therapy as first-line Helicobacter pylori treatment: a multicentre randomised trial in Japan. *Gut* 2020; 69: 1019–1026.
29. Chen PY, Wu MS, Chen CY, *et al.* Systematic review with meta-analysis: the efficacy of levofloxacin triple therapy as the first- or second-line treatments of Helicobacter pylori infection. *Aliment Pharmacol Ther* 2016; 44: 427–437.
30. Chuah SK, Liang CM, Lee CH, *et al.* A randomized control trial comparing two levofloxacin-containing second-line therapies for Helicobacter pylori eradication. *Medicine (Baltimore)* 2016; 95: e3586.
31. Rimbara E, Noguchi N, Kawai T, *et al.* Mutations in penicillin-binding proteins 1, 2 and 3 are responsible for amoxicillin resistance in Helicobacter pylori. *J Antimicrob Chemother* 2008; 61: 995–998.
32. Yang JC, Lin CJ, Wang HL, *et al.* High-dose dual therapy is superior to standard first-line or rescue therapy for Helicobacter pylori infection. *Clin Gastroenterol Hepatol* 2015; 13: 895–905.
33. Chey WD, Leontiadis GI, Howden CW, *et al.* ACG clinical guideline: treatment of Helicobacter pylori Infection [published correction appears in *Am J Gastroenterol* 2018; 113: 1102]. *Am J Gastroenterol* 2017; 112: 212–239.
34. Hsu PI, Chen WC, Tsay FW, *et al.* Ten-day quadruple therapy comprising proton-pump inhibitor, bismuth, tetracycline, and levofloxacin achieves a high eradication rate for Helicobacter pylori infection after failure of sequential therapy. *Helicobacter* 2014; 19: 74–79.
35. Shinozaki S, Kobayashi Y, Osawa H, *et al.* Effectiveness and safety of vonoprazan versus proton pump inhibitors for second-line *Helicobacter pylori* eradication therapy: systematic review and meta-analysis. *Digestion*. Epub ahead of print 8 January 2020. DOI: 10.1159/000504939.
36. Huang HT, Wang HM, Yang SC, *et al.* Efficacy of a 14-day quadruple-therapy regimen for third-line *Helicobacter pylori* eradication. *Infect Drug Resist* 2018; 11: 2073–2080.
37. Okimoto K, Arai M, Saito K, *et al.* Efficacy of levofloxacin based triple and high-dose PPI-amoxicillin dual eradication therapy for helicobacter pylori after failures of first- and second-line therapies. *Int Sch Res Notices* 2014; 2014: 631501.
38. Sue S, Shibata W, Sasaki T, *et al.* Randomized trial of vonoprazan-based versus proton-pump inhibitor-based third-line triple therapy with sitafloxacin for Helicobacter pylori. *J Gastroenterol Hepatol* 2019; 34: 686–692.
39. Saito Y, Konno K, Sato M, *et al.* Vonoprazan-based third-line therapy has a higher eradication rate against sitafloxacin-resistant Helicobacter pylori. *Cancers (Basel)* 2019; 11: 116.