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

Chih-Ming Liang, Wei-Chen Tai, Pin-I Hsu, Deng-Chyang Wu, Chao-Hung Kuo, Feng-Woey Tsay, Chia-Long Lee, Kuan-Yang Chen and Seng-Kee Chuah; on behalf of the Taiwan Acid-Related Disease and Microbiota Consortium Study Group

Abstract
Background: Antibiotic resistance plays a crucial role in the treatment failure of Helicobacter 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 χ² 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. 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. However, the eradication rates dropped to unacceptable levels when levofloxacin-resistant strains existed. 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. The clinical decision for optimal eradication regimens should be supported by referring to the relative efficacies stratified by antibiotic resistance and safety profiles. 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. 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.

Statistical analysis
A χ² 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 χ² 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.

Figure 1. The trend of annual antibiotic resistance rates in the first-line treatment of *Helicobacter pylori* (n culture = 1369).
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. Metronidazole resistance in *H. pylori* is relevant to the mutational inactivation of the redox-related gene (*frxA, rdxA*). Quinolone resistance is determined in *H. pylori* (*N87* and *D91*) in the quinolone resistance-determining region of the *gyrA* gene of *H. pylori*. Amoxicillin resistance is associated with alterations in penicillin-binding proteins. The triple base pair 16S rDNA mutation of AGA (926–928) to TTC mediates high-level tetracycline resistance in *H. pylori*. 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 treatment.
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

Figure 3. The trend of annual antibiotic resistance rates in the third-line treatment of *Helicobacter pylori* (*n* culture = 184).
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 systematic 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 (*PBPI, 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. systematic 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; \(p=0.006\)).\(^3\) 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.

**ORCID iDs**
Chih-Ming Liang \(\text{https://orcid.org/0000-0002-6614-5937}\)
Deng-Chyang Wu \(\text{https://orcid.org/0000-0003-3742-0634}\)
Seng-Kee Chuah \(\text{https://orcid.org/0000-0002-8934-3223}\)

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