Journal of Microbiology, Immunology and Infection 56 (2023) 40—47

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

Original Article

Multiple amino acid substitutions in penicillin-binding protein-1A confer amoxicillin resistance in refractory *Helicobacter pylori* infection

Chia-Jung Kuo [a,b], Jun-Nong Ke [c], Tony Kuo [a,b], Cheng-Yu Lin [a,b], Sen-Yung Hsieh [a,b,c], Ya-Fang Chiu [c,e,f], Hui-Yu Wu [c], Mei-Zi Huang [c], Ngoc-Niem Bui [c,g], Cheng-Hsun Chiu [c,d,e], Cheng-Tang Chiu [a,b], Chih-Ho Lai [c,e,f,h,i,*]

[a] *Department of Gastroenterology and Hepatology, Chang Gung Memorial Hospital at Linkou, Taoyuan, Taiwan*
[b] *School of Medicine, College of Medicine, Chang Gung University, Taoyuan, Taiwan*
[c] *Graduate Institute of Biomedical Sciences, College of Medicine, Chang Gung University, Taoyuan, Taiwan*
[d] *Department of Pediatrics, Chang Gung Memorial Hospital at Linkou, Taoyuan, Taiwan*
[e] *Molecular Infectious Disease Research Center, Department of Laboratory Medicine, Chang Gung Memorial Hospital at Linkou, Taoyuan, Taiwan*
[f] *Department of Microbiology and Immunology, Research Center for Emerging Viral Infections, Chang Gung University, Taoyuan, Taiwan*
[g] *Faculty of Medicine, Can Tho University of Medicine and Pharmacy, Can Tho, Viet Nam*
[h] *Department of Medical Research, School of Medicine, China Medical University and Hospital, Taichung, Taiwan*
[i] *Department of Nursing, Asia University, Taichung, Taiwan*

Received 1 January 2022; received in revised form 6 June 2022; accepted 21 July 2022
Available online 6 August 2022

KEYWORDS
Refractory infection;
H. pylori;
Amoxicillin;

Abstract *Background:* Amoxicillin resistance in *Helicobacter pylori* is mainly associated with mutations in penicillin-binding protein-1A (PBP-1A). However, the specific amino acid substitutions in PBP-1A that confer amoxicillin resistance in *H. pylori* remain to be investigated. *Objective:* This study aimed to investigate the molecular mechanism underlying amoxicillin resistance in patients with refractory *H. pylori* infection.

* Corresponding author. Department of Microbiology and Immunology, College of Medicine, Chang Gung University, Taoyuan, Taiwan.
E-mail address: chlai@mail.cgu.edu.tw (C.-H. Lai).

https://doi.org/10.1016/j.jmii.2022.07.006

Resistance;
PBP-1A mutation

Methods: Esophagogastroduodenoscopy (EGD) was performed in patients with persistent *H. pylori* infection after at least two courses of *H. pylori* eradication therapy between January-2018 to March-2021. Refractory *H. pylori* was cultured from the gastric biopsy specimens. Antibiotic susceptibility testing was conducted to determine the minimum inhibitory concentrations (MICs). Sequence analysis of *pbp-1A* was performed for amoxicillin-resistant strains.
Results: Thirty-nine successfully cultured isolates were classified as refractory *H. pylori* isolates, and seventeen isolates were resistant to amoxicillin (MIC $>$ 0.125 mg/L). Sequence analysis of resistant strains showed multiple mutations in the C-terminal region of PBP-1A that conferred amoxicillin resistance in *H. pylori*. However, the number of PBP-1A mutations did not correlate with the high MICs of amoxicillin-resistant isolates. Notably, some amino acid substitutions were identified in all Taiwanese isolates with history of eradication failure but not in published amoxicillin-susceptible strains, suggesting that the mutations may play a role in conferring antibiotic resistance to these strains.
Conclusions: Our results show that amoxicillin resistance in refractory *H. pylori* is highly correlated with numerous PBP-1A mutations that are strain specific. Continuous improvements in diagnostic tools, particularly molecular analysis approaches, can help to optimize current antimicrobial regimens.

Introduction

Helicobacter pylori infection is associated with various gastrointestinal diseases, such as chronic gastritis, peptic ulcer disease, and gastric adenocarcinoma.[1] Proton pump inhibitors (PPI) and bismuth compounds combined with at least two antibiotics are considered the first-line treatment for *H. pylori* infection.[2] With increasing antibiotic use, the prevalence of *H. pylori* resistance has greatly increased, and a drop in the eradication rate after treatment has become a major concern worldwide.[3]

To overcome increased challenges in eradicating *H. pylori* infection, the Maastricht V/Florence Consensus Report recommends that antimicrobial susceptibility be determined for *H. pylori* isolates from cases in which first-line antibiotics fail.[4] Amoxicillin, a β-lactam antibiotic, is commonly used to treat *H. pylori* infection and is employed in nearly all eradication regimens.[5] The primary amoxicillin resistance rate is relatively low in most countries.[6] A meta-analysis of antimicrobial resistance in the Asia-Pacific region revealed that the primary resistance rate of amoxicillin was approximately 3%, compared with 20% and 21% for clarithromycin and levofloxacin, respectively.[7] Therefore, high-dose dual therapy using PPI and amoxicillin is an efficient strategy for first-line treatment of *H. pylori* infection. With this treatment, an eradication rate of 89.3% was achieved.[8]

Although *H. pylori* eradication rates using amoxicillin-containing regimens are high, the amoxicillin resistance rate of *H. pylori* has gradually increased.[9] Moreover, following eradication failure, higher rates of amoxicillin resistance are noted.[10] Because of the high amoxicillin resistance rate in *H. pylori* (13.1—34.1%) after repeated treatment,[10,11] the efficacy of high-dose dual therapy in advanced eradication treatment requires more comprehensive and prospective investigations. The resistance of Gram-negative bacteria to β-lactam antibiotics is closely related to β-lactamase activity. Although the *H. pylori* genome contains a β-lactamase-like gene, it has not shown to correlate with amoxicillin resistance.[12] Rather, amoxicillin resistance is mainly attributed to mutations in penicillin-binding protein-1A (PBP-1A).[13]

Culture-driven therapy is a cost-effective method for the treatment of refractory *H. pylori* infection and is indicated after two rounds of eradication failure, but availability is limited.[11] Genetic analysis can considerably reduce the time it takes to acquire antimicrobial susceptibility results.[14] However, most studies have focused on clarithromycin or levofloxacin resistance.[15] Very few studies have investigated the prevalence and role of amoxicillin resistance in *H. pylori* eradication failure after second-line and third-line treatments, or the corresponding molecular mechanisms involved in the development of refractory *H. pylori* infection. The present study investigated the occurrence of multiple amino acid substitutions in PBP-1A that conferred *H. pylori* resistance to amoxicillin.

Materials and methods

Patient recruitment and experimental design

This study was performed in Chang Gung Memorial Hospital, Linkou, Taiwan (a tertiary medical center with over 4000 beds) from January 2018 to March 2021. The inclusion criteria for recruiting subjects were as follows: patients over 20 years old, with refractory *H. pylori* infection, defined as chronic persistent *H. pylori* infection after two or more rounds of eradication therapies, and confirmed by positive results from the [^{13}C]-urea breath assay. On the other hand, the exclusion criteria included patients unwilling to undergo esophagogastroduodenoscopy (EGD), patients with underlying diseases (which is a contraindication to biopsies including cirrhosis, coagulopathy, or

thrombocytopenia), failure of *H. pylori* culture, and unavailability of antibiotic sensitivity test. All enrolled patients underwent EGD with biopsies of the stomach mucosa for subsequent *H. pylori* culture and molecular analysis to determine antimicrobial resistance.[10] A total of 70 patients were diagnosed as having refractory *H. pylori* infection, and 39 isolates were successfully cultured (Fig. 1). *H. pylori* isolates obtained from the stomach mucosa were then subjected to antibiotic susceptibility testing and molecular analysis, to investigate the mechanism of antimicrobial resistance. Patient characteristics and demographic data, including personal medical history, previous antibiotic exposure, endoscopic findings, biopsy pathological reports, eventual antibiotic prescription, and the results of eradication, were recorded.

Ethics

All recruited patients have signed the informed consent to participate in the study protocol after providing a complete explanation of the study. The gastric biopsies and clinical data were acquired after obtaining patients' consent. All the clinical protocols were in accordance with the guidelines approved by the Institutional Review Board (IRB) of Chang Gung Memorial Hospital, Linkou, Taiwan (IRB No. 201701000A3 and 202000374A3) and the ethical regulation of the hospital.

Isolation of *H. pylori* strains and identification

The biopsies isolated from both gastric antrum and body were incubated at 37 °C in the microaerophilic environment for 5—7 days. *H. pylori* isolates were identified as described previously.[16] The *H. pylori* isolates were stored at −80 °C before experimental studies were conducted.

Antibiotic susceptibility testing

Epsilometer test (E-test) (AB Biodisc, Solna, Sweden) was used to determine the antimicrobial susceptibility for all *H. pylori* isolates. The breakpoint of *H. pylori* amoxicillin-resistance according to EUCAST (https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_10.0_Breakpoint_Tables.pdf, accessed on December 20th, 2021) was defined as 0.125 mg/L. After *H. pylori* was cultured for 2—3 days, the minimal inhibitory concentration (MIC) value was analyzed and defined as described previously.[17]

Polymerase chain reaction (PCR) analysis and DNA sequencing

H. pylori genomic DNA was prepared by following the manufacturer's instructions (Promega, Dane, WI, USA). Amplification of C-terminal region (992-1896 bae pairs) in *pbp-1A* was performed using PCR with forward and reverse primers: PBP1-F (GCATGATCGTTACAGACACG) and PBP1-R (ATCCACGATTTCTTTACGC), respectively.[18] The PCR cycling reaction for *pbp-1A* is: an initial denaturation at 95 °C for 5 min; then 95 °C for 1 min, 52 °C for 1 min, 72 °C for 1.5 min for a total of 30 cycles; and a final extension at 72 °C for 10 min. The PCR fragments with amplicon of 905 base pairs were prepared and sequenced. The mutation sites of *pbp-1A* were determined by DNA sequencing and compared to *H. pylori* reference strain ATCC 700392.[19]

Statistical analysis

Analysis of the correlation of number of amino acid mutations in *pbp-1A* of *H. pylori* isolates and MIC value were conducted using SPSS version 22 (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

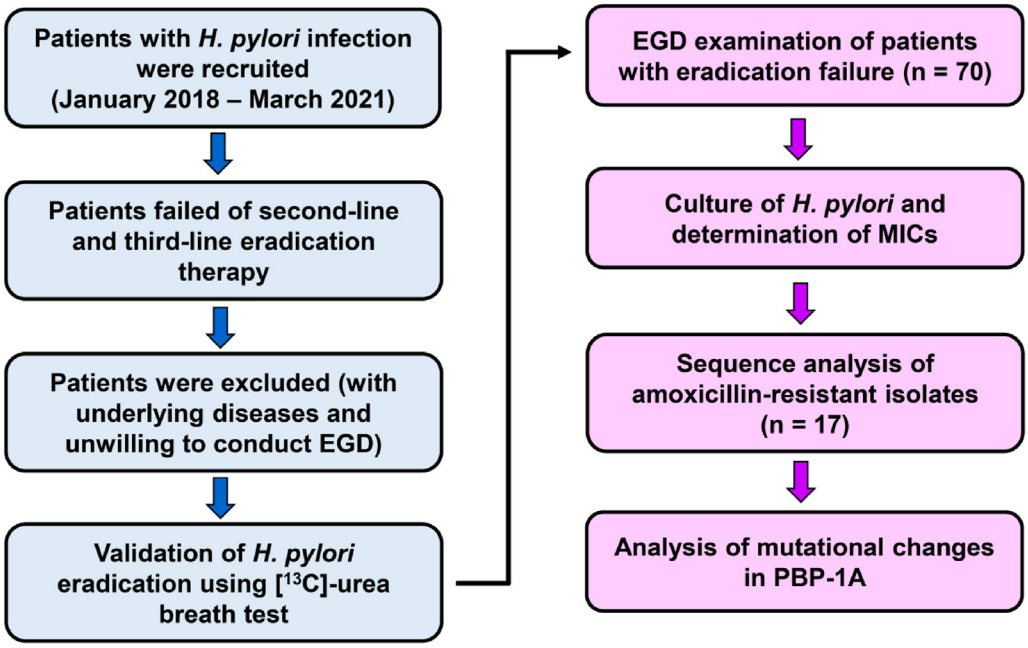

Figure 1. Patient recruitment and experimental protocol of the study.

Results

Identification of amoxicillin-resistant *H. pylori* isolates

After completion of the antibiotic-based therapy course, *H. pylori* eradication status of each patient was assessed using the [^{13}C]-urea breath test. A total of 70 patients showed failure of *H. pylori* eradication, and the corresponding *H. pylori* strains were cultured. Patients who tested positive for *H. pylori* using the [^{13}C]-urea breath test but did not have a successful bacterial culture were not considered for further analysis. Of the 70 patients with treatment failure, only 39 had positive cultures, and the corresponding isolates were referred to as those obtained from refractory *H. pylori* infections. We then analyzed the antibiotic susceptibility of all *H. pylori* isolates using the E-test. Among these, 17 isolates, with an amoxicillin MIC higher than 0.125 mg/L were considered to have acquired an amoxicillin resistance phenotype. The MIC values for amoxicillin-resistant *H. pylori* strains ranged from 0.19 to 256 mg/L (Table 1). In the amoxicillin-resistant isolates, 8 (47.1%), 3 (17.6%), and 6 (35.3%) had MICs of 0.125—0.5 mg/L, 0.5—1.0 mg/L, and >1.0 mg/L, respectively.

Among the 17 patients infected with amoxicillin-resistant *H. pylori* strains, 64.7% were female with a mean age of 52.7 ± 12.7 years. Endoscopic analyses revealed gastritis (13/17, 76.5%) and duodenal ulcer scarring (6/17, 35.3%) to be the most common abnormalities, and we established that the median course of previous antibiotic treatment for *H. pylori* eradication was three (Table 2).

Table 1 Amino acid substitutions in PBP-1A of *H. pylori* strains.

	H. pylori isolate†																					
	Susceptible					Resistance																
	16-Amx-S-C3¶	1061§	4	6	21	62A	40A	41A	47B	43B	5	20	64B	53B	18	44A	57B	48A	50A	51A	55B	60A
MIC (mg/L)	<0.016	<0.016	<0.016	<0.016	<0.016	0.19	0.23	0.25	0.25	0.32	0.38	0.38	0.5	0.64	0.75	1	1.25	1.5	>256	>256	>256	>256
K107	R	R																				
F125	L	L																				
K352										I												
K363												T										
F366															L							
G367												A										
A369								T														
V374								L					L								L	
Q376										P												
T386										P												
F396										S		S										
H409								R														
S414															R							
R418																					H	
E448											K			K								
V469			L									M						L				
F473															V				V			
A474				T	T		V				V			V		T						
D479			E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
N504	D	D	D	D	D	D		D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
D508							E					N			N				N			
V509												I			I				I			
T511					I																	
T513						M		M	M	M			M			M			M	M		
M515	I	I	I		I																	
L530	I	I																I				
D535	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
T541										P												
G542					E																	
S543							N					R			R							
T550							S											S				
N561											D			D								
I563			V	T	V																	
S589	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G
G591								R					R								R	
T593			A		A			S			S		S	S		G		A			S	
G595			S			S		S			S		S	S		S					S	S
Y604																	H					
S615																						L
K617												I										
R618																						K
F620																						V
V622																						C
P623						L	L	L	L	L	L	L	L	L	L	L	L			L		
T644	I	I																				
K648	Q	Q																				

Amino acid position and substitution of PBP1A‡

†Green and purple table elements represent refractory isolates that are susceptible and resistant to amoxicillin, respectively.
‡Red table elements indicate the observation of same mutational changes in all isolates. Yellow table elements indicate amino acid mutations in amoxicillin-susceptible strains. Blue table elements indicate mutation changes in amoxicillin-resistant strains.
¶Amoxicillin-susceptible *H. pylori* isolate (GenBank accession numbers EF583173).[31]
§Amoxicillin-susceptible *H. pylori* isolate (GenBank accession numbers AF479618).[32]

Table 2 Characteristics of patients recruited in the study.

Patient's characteristics		Amoxicillin resistance (n = 17)
Gender	Male	6 (35.3%)
	Female	11 (64.7%)
Age in years, mean ± SD		52.7 ± 12.7
Age		
30-39		4 (23.5%)
40-49		4 (23.5%)
50-59		2 (11.8%)
60-69		5 (29.4%)
70-79		2 (11.8%)
Background definition		
1. Gastritis		13 (76.5%)
2. Erosion		1 (5.9%)
3. Gastric ulcer		3 (17.6%)
4. Duodenal ulcer scar		6 (35.3%)
Median previous antibiotic courses		3
Mean previous antibiotic courses		3.8

Analysis of PBP-1A mutations in amoxicillin-resistant *H. pylori*

In *H. pylori*, mutations in the *pbp-1A* gene confer resistance to amoxicillin.[20,21] The *pbp-1A* gene of 3 amoxicillin-sensitive and 17 amoxicillin-resistant isolates was amplified using PCR for DNA sequencing (Fig. 2A). Two published amoxicillin-susceptible strains, 16-Amx-S-C3 and 1061, were also analyzed. Compared to the reference strain (ATCC 700392), there were 12 and 38 amino acid substitutions in PBP-1A of amoxicillin-susceptible and -resistant isolates, respectively (Table 1). Four mutations (T511, M515, G542, and I563) were observed only in the sensitive strains analyzed in this study, suggesting that they do not correlate with amoxicillin resistance. On the other hand, there were 30 substitutions in PBP-1A (K352, K363, F366, G367, A369, V374, Q376, T386, F396, H409, S414, R418, F448, F473, D508, V509, T513, L530, T541, S543, T550, N561, G591, Y604, S615, K617, R618, F620, V622, and P623) that were only detected in amoxicillin-resistant isolates. We noticed that most resistant isolates (except strains 48A, 58A, 55B, and 60A) carried the P623L substitution, which is likely responsible for the development of amoxicillin resistance. Importantly, three mutations (D479E, D535N, and S589G) were identified across all isolates from Taiwanese patients. Additionally, the number of mutations in PBP-1A did not correlate with the high MICs of amoxicillin-resistant isolates (Fig. 2B).

Discussion

In addition to antibiotic resistance, many factors may contribute to the failure of *H. pylori* eradication therapy, including bacterial genotypes, polymorphisms in genes associated with metabolism of PPIs, intragastric acidity, high bacterial load before treatment, and patient compliance.[22] The Maastricht V/Florence Consensus Report suggests that after one or two failed treatments with different antibiotics, *H. pylori* culture and standard antibiotic sensitivity tests should be performed.[4] In fact, in the treatment of refractory *H. pylori* infection, antimicrobial susceptibility testing and genotypic resistance-guided therapy are more effective than empirical therapy.[23] However, both bacterial culture and antibiotic sensitivity tests are laborious and time-consuming because of their fastidious nature. Additionally, the culture success rate of *H. pylori* isolated from infection biopsies is not that high, ranging from 50% to 70%.[24] As the eradication

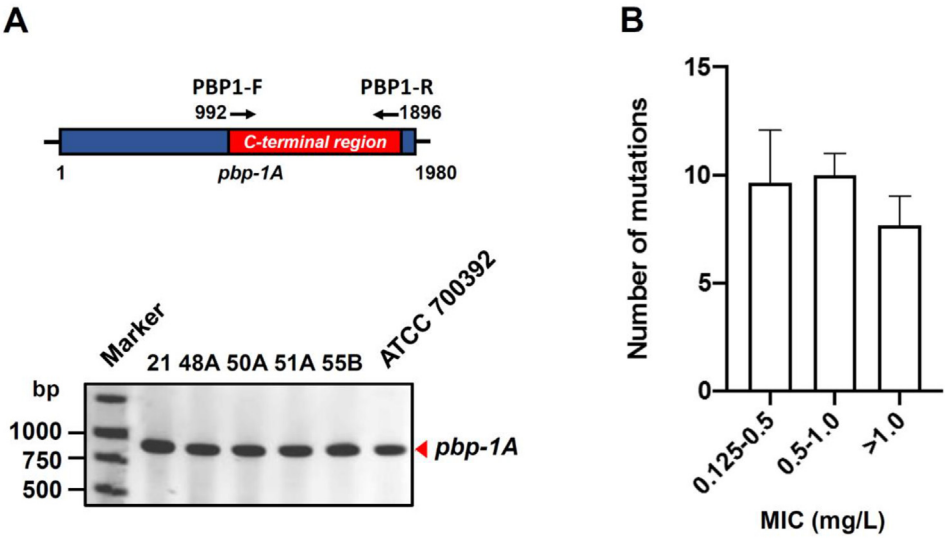

Figure 2. Analysis of amino acid mutations in PBP-1A. (A) Forward and reverse primers in C-terminal region of *pbp-1A* (992—1896 bp) (upper panel), and PCR amplicon of 905 bp, representing *pbp-1A* (lower panel). (B) Relationship between the number of mutational changes in PBP-1A and MIC. PBP-1A, penicillin-binding protein-1A; PCR, polymerase chain reaction; MIC, minimum inhibitory concentration.

rate decreases, the need for antimicrobial susceptibility testing increases, but its use remains limited in clinical practice. This study reports the molecular mechanism of amoxicillin resistance in *H. pylori* isolates, which can help identify appropriate antimicrobial agents to achieve better treatment outcomes.

Clarithromycin resistance is the main cause of *H. pylori* eradication failure following standard triple therapy.[6] It is recommended that standard triple therapies should not be used in areas with clarithromycin resistance rates higher than 20%, because the eradication rates of standard triple therapies are generally less than 80%.[25] However, the primary amoxicillin resistance rate of *H. pylori* is often low; thus, it is advantageous to use amoxicillin to treat *H. pylori* infections.[7] In tandem with the gradual increase in amoxicillin use over several decades, amoxicillin resistance in *H. pylori* strains has increased globally after unsuccessful eradication therapy.[6] After failure of therapy in patients who were previously subjected to *H. pylori* eradication treatment, amoxicillin resistance has increased in many countries, with resistance rates of approximately 3.48%, 8.1%, and 9.5% in Poland,[26] South Korea,[27] and Vietnam,[28] respectively. Recently, we found the amoxicillin resistance rate in Taiwanese patients with *H. pylori* eradication failure after at least two courses of treatment to be as high as 34.1%.[10] These findings indicate that an increase in amoxicillin use directly impacts the development of amoxicillin-resistant *H. pylori*, resulting in increased treatment failure. Therefore, a precise approach to identify *H. pylori* antimicrobial resistance during diagnosis is essential to determine which antibiotics can effectively treat patients.

Amoxicillin is frequently used in eradication therapy for *H. pylori* infections. Penicillin-binding proteins (PBPs) are involved in biosynthesis of the bacterial peptidoglycan layer. Amoxicillin binds to PBPs in the periplasmic space, affecting transpeptidase activity that is required for cross-linking of nascent peptidoglycan molecules; this, in turn, interferes with stability of the cell wall and ultimately leads to bacterial lysis.[13] *H. pylori* resistance to amoxicillin is thought to be caused by mutations in the *pbp-1A* gene. PBPs with structural mutations exhibit reduced affinity for β-lactam antibiotics.[29] These mutations protect bacteria from antibiotic activity and allow bacteria to survive, despite high concentrations of antimicrobial agents in cells. However, there is a paucity of known amoxicillin resistance genotype profiles. *H. pylori* resistance to amoxicillin has been induced gradually, and higher resistance rates to amoxicillin have emerged after eradication failures.[6,10] Notably, antimicrobial treatment not only increases specific resistance to the antibiotics used for bacterial eradication therapy but also increases resistance to other types of antibiotics.[30] Our study provides a molecular approach for the detection of *H. pylori* resistance to amoxicillin, which can help physicians further optimize currently available regimens.

Analysis of PBP-1A sequences of two published amoxicillin-susceptible strains, Amx-S-C3[31] and 1061,[32] revealed that both carried same substitutions in 9 amino acids, suggesting that these substitutions were not associated with amoxicillin resistance. Qureshi et al. reported that six sites in PBP1 are critical for amoxicillin susceptibility: three amino acid substitutions in PBP1 (V469M, F473L, and S543R) affected the susceptibility of the US clinical strain,[29] while the other three substitutions (S414R, T556S, and N562Y) have been reported in multiple clinical isolates, indicating that these are the most common amino acid changes in PBP1 associated with amoxicillin resistance.[20,21] Alteration of amino acids S414R and F473L has been posited to change the protein structure around the putative amoxicillin binding cleft.[32] In addition, amino acid substitutions in, or adjacent to, putative penicillin-binding motifs (T556S and N562Y) affect amoxicillin susceptibility.[33] Our study showed that several substitutions in PBP-1A, such as S414, V469, F473, and S543, present in amoxicillin-resistant isolates were similar to those in the aforementioned US study.[29] Additionally, S414 mutations have been reported in the Netherlands[32] and South Korea.[34] Substitutions including V374, S414, and V509 were observed in amoxicillin-resistant strains evaluated in a study in Japan.[18] Similarly, the substitutions V374 and S414 have been reported in amoxicillin-resistant strains isolated from Mongolian population.[35] These results indicate that multiple mutations, specifically those located in the C-terminal region of PBP-1A, confer amoxicillin resistance and facilitate refractory *H. pylori* infection.

In the present study, three amino acid substitutions, D479E, D535N, and S589G, were found in all amoxicillin-resistant *H. pylori* isolates. However, the three mutations appeared either alone or along with amino acid substitutions associated with amoxicillin-resistant *H. pylori* reported in other countries.[18,20] Tseng et al. previously reported that *H. pylori* with high-level amoxicillin resistance isolated from a patient in southern Taiwan had amino acid substitutions, including D479E, D535N, and S589G.[36] The present study involved the analysis of amoxicillin-resistant *H. pylori* isolates from patients in northern Taiwan, and still all strains exhibited these three mutational changes. These findings indicate that isolates from cases in the Taiwanese population with a history of eradication failure may possess geographic uniqueness and that specific mutations appear to be involved in conferring antibiotic resistance. Our results can be explained by previous studies showing that *H. pylori*-infected populations have distinct geographical distributions.[37]

Although the current study elucidated specific amino acid substitutions in PBP-1A associated with amoxicillin resistance in *H. pylori*, some limitations of this study should be considered. First, the breakpoint for amoxicillin resistance was different than those used in other studies; EUCAST proposed 0.125 mg/L as the breakpoint for amoxicillin resistance,[38] but some studies used an MIC of 0.5 mg/L for resistance to amoxicillin.[39] Second, the E-test is commonly used to assess the antimicrobial susceptibility of *H. pylori*; additional analyses, including agar dilution or disc diffusion tests, are recommended to validate the MIC breakpoint of the antibiotics studied. Third, the current study was a single-centered study, and the sample size was not large. Given the apparent low incidence of *H. pylori* amoxicillin resistance in the Taiwanese population and that *H. pylori* is notoriously difficult to culture, particularly from samples collected from patients who have undergone repeated antibiotic treatment,[17,40—42] obtaining sufficiently large sample sizes is a current challenge. Fourth, bacterial virulence factors were not analyzed in the present study. In addition to PBP-1A mutations, there are other

possible causes of amoxicillin-based eradication failure, including activation of efflux pumps and alteration of membrane permeability.[6] Complete genome sequencing to identify all mutations that may be involved in encoding PBPs that confer *H. pylori* resistance is required. Finally, host factors such as drug adherence and host genetic polymorphisms in genes associated with PPI metabolism are also contributors to therapy failure. Therefore, further studies on the interplay between mechanistic processes associated with *H. pylori* resistance and host factors that lead to eradication failure are warranted.

In summary, this study showed that amoxicillin resistance in our *H. pylori* isolates correlated with the presence of multiple mutations in the C-terminal region of PBP-1A. Using molecular approaches, resistant clinical *H. pylori* strains can be rapidly identified and amino acid substitutions conferring antibiotic resistance in *H. pylori* can be precisely determined. Improvements in diagnostic tools will help to optimize current antimicrobial regimens, leading to a reduction in the occurrence of *H. pylori* therapy failure, in addition to an increase in eradication rates.

Author contributions

Conceived the project: C.-J.K., C.-T.C., C.-H.L.
Designed the research: C.-J.K., C.-H.C., C.-H.L.
Performed the studies: J.-N.K., T.K., C.-Y.L., H.-Y.W., N.-N.B.
Interpreted the results: J.-N.K., T.K., C.-Y.L., S.-Y.H., H.-Y.W.
Drafted the manuscript: C.-J.K., J.-N.K., Y.-F. C., C.-H.L.
Final approval of the manuscript: all authors.

Funding

This work was funded by the National Science and Technology Council, Taiwan (109-2320-B-182-029-MY3 and 109-2622-B-182-002), and Chang Gung Memorial Hospital, Linkou, Taiwan (CMRPG3F0551-2, CMRPG3H0031, CMRPG3H0032, CMRPG3K0691, and CPRPG3M0031).

Declaration of competing interest

The authors declared that this study was performed in the absence of a potential conflict of interest.

Acknowledgments

The authors would like to thank the editor and reviewers for the editorial assistance and their valuable comments. The authors sincerely appreciate the assistance from Miss Ning-En Hu for the clinical isolation of *H. pylori*.

References

1. Suerbaum S, Michetti P. *Helicobacter pylori* infection. *N Engl J Med* 2002;**347**:1175—86.
2. Liou JM, Malfertheiner P, Lee YC, Sheu BS, Sugano K, Cheng HC, et al. Screening and eradication of Helicobacter pylori for gastric cancer prevention: the Taipei global consensus. *Gut* 2020;**69**:2093—112.
3. Zou Y, Qian X, Liu X, Song Y, Song C, Wu S, et al. The effect of antibiotic resistance on Helicobacter pylori eradication efficacy: a systematic review and meta-analysis. *Helicobacter* 2020;**25**:e12714.
4. Malfertheiner P, Megraud F, O'Morain CA, Gisbert JP, Kuipers EJ, Axon AT, et al. Management of *Helicobacter pylori* infection-the Maastricht V/florence consensus report. *Gut* 2017;**66**:6—30.
5. Gao CP, Zhang D, Zhang T, Wang JX, Han SX, Graham DY, et al. PPI-amoxicillin dual therapy for Helicobacter pylori infection: an update based on a systematic review and meta-analysis. *Helicobacter* 2020;**25**:e12692.
6. Thung I, Aramin H, Vavinskaya V, Gupta S, Park JY, Crowe SE, et al. Review article: the global emergence of *Helicobacter pylori* antibiotic resistance. *Aliment Pharmacol Ther* 2016;**43**:514—33.
7. Kuo YT, Liou JM, El-Omar EM, Wu JY, Leow AHR, Goh KL, 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—15.
8. Yang JC, Lin CJ, Wang HL, Chen JD, Kao JY, Shun CT, 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. e5.
9. Alba C, Blanco A, Alarcon T. Antibiotic resistance in *Helicobacter pylori*. *Curr Opin Infect Dis* 2017;**30**:489—97.
10. Kuo CJ, Lee CH, Chang ML, Lin CY, Lin WR, Su MY, et al. Multidrug resistance: the clinical dilemma of refractory *Helicobacter pylori* infection. *J Microbiol Immunol Infect* 2021;**54**:1184—7.
11. Liou JM, Chen CC, Chang CY, Chen MJ, Fang YJ, Lee JY, et al. Efficacy of genotypic resistance-guided sequential therapy in the third-line treatment of refractory Helicobacter pylori infection: a multicentre clinical trial. *J Antimicrob Chemother* 2013;**68**:450—6.
12. Tshibangu-Kabamba E, Ngoma-Kisoko PJ, Tuan VP, Matsumoto T, Akada J, Kido Y, et al. Next-generation sequencing of the whole bacterial genome for tracking molecular insight into the broad-spectrum antimicrobial resistance of Helicobacter pylori clinical isolates from the democratic Republic of Congo. *Microorganisms* 2020;**8**.
13. Tshibangu-Kabamba E, Yamaoka Y. Helicobacter pylori infection and antibiotic resistance - from biology to clinical implications. *Nat Rev Gastroenterol Hepatol* 2021;**18**:613—29.
14. Lauener FN, Imkamp F, Lehours P, Buissonnière A, Benejat L, Zbinden R, et al. Genetic determinants and prediction of antibiotic resistance phenotypes in Helicobacter pylori. *J Clin Med* 2019;**8**.
15. Liou JM, Chang CY, Sheng WH, Wang YC, Chen MJ, Lee YC, et al. Genotypic resistance in Helicobacter pylori strains correlates with susceptibility test and treatment outcomes after levofloxacin- and clarithromycin-based therapies. *Antimicrob Agents Chemother* 2011;**55**:1123—9.
16. Du SY, Wang HJ, Cheng HH, Chen SD, Wang LH, Wang WC. Cholesterol glucosylation by *Helicobacter pylori* delays internalization and arrests phagosome maturation in macrophages. *J Microbiol Immunol Infect* 2016;**49**:636—45.
17. Kuo CJ, Lin CY, Le PH, Chang PY, Lai CH, Lin WR, et al. Rescue therapy with rifabutin regimen for refractory *Helicobacter pylori* infection with dual drug-resistant strains. *BMC Gastroenterol* 2020;**20**:218.
18. Kageyama C, Sato M, Sakae H, Obayashi Y, Kawahara Y, Mima T, et al. Increase in antibiotic resistant *Helicobacter pylori* in a University hospital in Japan. *Infect Drug Resist* 2019;**12**:597—602.
19. Tomb JF, White O, Kerlavage AR, Clayton RA, Sutton GG, Fleischmann RD, et al. The complete genome sequence of the

gastric pathogen *Helicobacter pylori*. *Nature* 1997;**388**: 539—47.
20. Gerrits MM, Godoy AP, Kuipers EJ, Ribeiro ML, Stoof J, Mendonca S, et al. Multiple mutations in or adjacent to the conserved penicillin-binding protein motifs of the penicillin-binding protein 1A confer amoxicillin resistance to *Helicobacter pylori*. *Helicobacter* 2006;**11**:181—7.
21. Kwon DH, Dore MP, Kim JJ, Kato M, Lee M, Wu JY, et al. High-level beta-lactam resistance associated with acquired multidrug resistance in *Helicobacter pylori*. *Antimicrob Agents Chemother* 2003;**47**:2169—78.
22. Graham DY, Fischbach L. Helicobacter pylori treatment in the era of increasing antibiotic resistance. *Gut* 2010;**59**:1143—53.
23. Liou JM, Chen PY, Luo JC, Lee JY, Chen CC, Fang YJ, et al. Efficacies of genotypic resistance-guided vs empirical therapy for refractory *Helicobacter pylori* infection. *Gastroenterology* 2018;**155**:1109—19.
24. Miftahussurur M, Yamaoka Y. Diagnostic methods of Helicobacter pylori infection for epidemiological studies: critical importance of indirect test validation. *BioMed Res Int* 2016; **2016**:4819423.
25. Chey WD, Leontiadis GI, Howden CW, Moss SF. ACG clinical guideline: treatment of Helicobacter pylori infection. *Am J Gastroenterol* 2017;**112**:212—39.
26. Szadkowski A, Zemlak M, Muszynski J. Effectiveness of *Helicobacter pylori* eradication established on the basis of examination of antibiotic resistance of the bacteria. *Przeglad Gastroenterol* 2018;**13**:93—8.
27. Lee JY, Kim N, Nam RH, Choi S, Lee JW, Lee DH. Primary and secondary antibiotic resistance of *Helicobacter pylori* in Korea from 2003 to 2018. *Helicobacter* 2019;**24**:e12660.
28. Khien VV, Thang DM, Hai TM, Duat NQ, Khanh PH, Ha DT, et al. Management of antibiotic-resistant *Helicobacter pylori* infection: perspectives from Vietnam. *Gut Liver* 2019;**13**: 483—97.
29. Qureshi NN, Morikis D, Schiller NL. Contribution of specific amino acid changes in penicillin binding protein 1 to amoxicillin resistance in clinical *Helicobacter pylori* isolates. *Antimicrob Agents Chemother* 2011;**55**:101—9.
30. Zimmermann P, Curtis N. The effect of antibiotics on the composition of the intestinal microbiota - a systematic review. *J Infect* 2019;**79**:471—89.
31. Matteo MJ, Granados G, Olmos M, Wonaga A, Catalano M. *Helicobacter pylori* amoxicillin heteroresistance due to point mutations in PBP-1A in isogenic isolates. *J Antimicrob Chemother* 2008;**61**:474—7.
32. Gerrits MM, Schuijffel D, van Zwet AA, Kuipers EJ, Vandenbroucke-Grauls CM, Kusters JG. Alterations in penicillin-binding protein 1A confer resistance to beta-lactam antibiotics in *Helicobacter pylori*. *Antimicrob Agents Chemother* 2002;**46**:2229—33.
33. Rimbara E, Noguchi N, Kawai T, Sasatsu M. Correlation between substitutions in penicillin-binding protein 1 and amoxicillin resistance in *Helicobacter pylori*. *Microbiol Immunol* 2007;**51**: 939—44.
34. Kim BJ, Kim JG. Substitutions in penicillin-binding protein 1 in amoxicillin-resistant *Helicobacter pylori* strains isolated from Korean patients. *Gut Liver* 2013;**7**:655—60.
35. Azzaya D, Gantuya B, Oyuntsetseg K, Davaadorj D, Matsumoto T, Akada J, et al. High antibiotic resistance of *Helicobacter pylori* and its associated novel gene mutations among the Mongolian population. *Microorganisms* 2020;**8**.
36. Tseng YS, Wu DC, Chang CY, Kuo CH, Yang YC, Jan CM, et al. Amoxicillin resistance with beta-lactamase production in *Helicobacter pylori*. *Eur J Clin Invest* 2009;**39**:807—12.
37. Moodley Y, Linz B, Yamaoka Y, Windsor HM, Breurec S, Wu JY, et al. The peopling of the Pacific from a bacterial perspective. *Science* 2009;**323**:527—30.
38. Chen MJ, Wu MS, Chen CC, Chen CC, Fang YJ, Bair MJ, et al. Impact of amoxicillin resistance on the efficacy of amoxicillin-containing regimens for *Helicobacter pylori* eradication: analysis of five randomized trials. *J Antimicrob Chemother* 2017;**72**:3481—9.
39. Hsueh PR, Ko WC, Wu JJ, Lu JJ, Wang FD, Wu HY, et al. Consensus statement on the adherence to clinical and laboratory standards institute (CLSI) antimicrobial susceptibility testing guidelines (CLSI-2010 and CLSI-2010-update) for enterobacteriaceae in clinical microbiology laboratories in taiwan. *J Microbiol Immunol Infect* 2010;**43**:452—5.
40. Liang CM, Tai WC, Hsu PI, Wu DC, Kuo CH, Tsay FW, et al. Trend of changes in antibiotic resistance in *Helicobacter pylori* from 2013 to 2019: a multicentre report from Taiwan. *Therap Adv Gastroenterol* 2020;**13**:1756284820976990.
41. Huang TH, Yang SC, Tai WC, Liang CM, Kuo CM, Yao CC, et al. The efficacies of non-bismuth containing quadruple therapies in the treatment of first-line anti-*Helicobacter pylori* across 4-year time interval with changing antibiotics resistance. *Biomed J* 2021;**44**:S275—81.
42. Su DJ, Chang MH, Yang JC, Ni YH, Hsu HY, Wu JF. Fourteen-day sequential therapy is superior to 7-day triple therapy as first-line regimen for *Helicobacter pylori* infected children. *J Formos Med Assoc* 2022;**121**:202—9.