

RESEARCH ARTICLE

High Fluoroquinolone Resistant Strains of *Helicobacter pylori* in the Golden triangle

Ratha korn Vilaichone^{1,8*}, Thawee Ratanachu ek^{2,8}, Pornpen Gamnarai^{3,8}, Phawinee Subsomwong⁴, Tomahisa Uchida⁵, Yoshio Yamaoka^{4,6}, Varocha Mahachai^{7,8}

Abstract

Background and aims: *Helicobacter pylori* (*H. pylori*) infections, associated with fatal GI diseases such as gastric cancer and MALT lymphoma, remain a major health problem in ASEAN countries. The Golden triangle has long been known as one of Asia's main opium-producing areas. There have been no prior studies of *H. pylori* infection in this area. The major objectives of this project were therefore to establish prevalence, antibiotic resistance patterns and associated predictive in the Golden triangle. **Methods:** We recruited dyspeptic patients in Chiang khong and Chiang saen districts, Chiangrai province of Thailand. All subjects underwent gastroscopy, and 3 antral gastric biopsies were collected for rapid urease tests and *H. pylori* culture. E-tests were used to evaluate the MICs for metronidazole (MNZ), levofloxacin (LVX), ciprofloxacin (CIP), amoxicillin (AMX), tetracycline (TET) and clarithromycin (CLR). **Results:** Total of 148 patients was included. *H. pylori* infection was present in 36.3% (37/102) of Chiang khong and 34.8 % (16/46) of Chiang saen subjects and the overall *H. pylori* infection rate was 35.8% (53/148). Antibiotic resistance was demonstrated in 44%, including 2% for CLR and 26% for MNZ, whereas fluoroquinolone resistance was demonstrated to be as high as 25% in Chiang khong. Multi-drug resistant *H. pylori* was detected in 4%. There was no AMX and TET resistance in this study. The prevalence of CLR resistance on a background of gastritis was significantly higher than peptic ulcer disease in the golden triangle area (100% vs 0%; P= 0.04). **Conclusions:** *H. pylori* remains a common infection in the Golden triangle. MNZ resistance appears to be high, whereas fluoroquinolone resistance is prevalent and is becoming a significant problem in this area. Diagnosis of gastritis might be a predictor of CLR resistance in the Golden triangle. *H. pylori* eradication with an appropriate regimen by using the local antibiotic resistant pattern is a key important tool to reduce *H. pylori* associated GI diseases in this particular part of the world.

Keywords: *Helicobacter pylori*- antibiotic resistance- Golden triangle

Asian Pac J Cancer Prev, **18** (2), 455-458

Introduction

Helicobacter pylori (*H. pylori*) is a common bacterial infection in world population. *H. pylori* causes a chronic infection and is the major cause of fatal GI diseases such as gastric cancer and MALT lymphoma. (Vilaichone et al., 2001; Vilaichone et al., 2005; Vilaichone et al., 2006; Vilaichone et al., 2016). The prevalence of gastric cancer varies among Asian countries; for instance, its prevalence is high in Japan, Korea, and China but low in India and Indonesia. In Thailand, gastric cancer is the sixth most common cancer in males and ninth in females with annual incidence of 5 cases per 100,000 people. Most Thai gastric cancer patients presented in advance stage who had a grave prognosis. *H. pylori* eradication is considered as an

effective prevention for this particular cancer (Vilaichone et al., 2014). The proper eradication regimens definition should be relied on local antibiotic resistant pattern in individual area. In Thailand, we detected MNZ resistant strains approximately 40-50% (Vilaichone et al., 2013, Vilaichone et al., 2015); whereas, CLR resistance was up to 14% .

Golden triangle is a long famous historical place and was the main opium-producing areas in Asia. This particular area has approximately 950,000 square kilometres (367,000 square miles) which included three mountains of Thailand, Laos, and Myanmar. There have been different views of the evolution of the term 'Golden triangle' and even disagree on when the term was coined. Mostly agree that this name might be related to opium

¹Gastroenterology Unit, Department of Medicine, ²Department of Biochemistry, Thammasat University Hospital, ³National Gastric Cancer and Gastrointestinal Diseases Research Center, Pathumthani, ⁴Department of Surgery, Rajavithi Hospital, ⁵GI and Liver Center, Bangkok Medical Center (BMC), Bangkok, Thailand, ⁶Department of Environmental and Preventive Medicine, ⁷Department of Molecular Pathology, Oita University Faculty of Medicine, Hasama-Machi, Yufu-City, Oita, Japan, ⁸Department of Medicine-Gastroenterology, Baylor College of Medicine and Michael E. DeBakey Veterans Affairs Medical Center, Houston, Texas, United States of America. *For Correspondence: Vilaichone@hotmail.co.th

producing region including Northern Thailand, Western Laos, and Eastern Myanmar. The prevalence and drug resistant pattern of *H. pylori* infection have never been reported before from this area. The objectives of this study was to survey the prevalence of *H. pylori* infection, assess antibiotic resistance patterns and determine the predictive factors of antibiotic resistant *H. pylori* in the “Golden triangle” and would be used this information for health care planning to reduce *H. pylori* associated diseases in this particular area.

Matherials and Methods

In January 2016, we started a community-based endoscopic survey among dyspeptic patients in Chiang khong and Chiang saen districts, Chiangrai province, Golden triangle area in Thailand (Figures 1 and 2). All participants were people living in the community during our survey period. Patients aged more than 18 years with dyspeptic symptoms who never received *H. pylori* eradication, or proton pump inhibitor (PPI), H2-blocker, bismuth compound or antimicrobial agents during the previous month were excluded. We also excluded patients who received anticoagulants such as coumadin, patients with severe systemic illnesses, alcoholism or drug abuse. Gastrosopies were performed by our GI endoscopists. Three antral gastric biopsies were conducted on all patients and placed into the transport media tubes. Tubes were kept in our mobile freezer unit and immediately sent back to the culture rooms at Thammasat University Hospital, Pathumthani, Thailand. The diagnosis of peptic ulcer was done based upon endoscopic findings. Dyspeptic patients with normal endoscopy or mild inflammation were counted as gastritis. *H. pylori* infection was defined as positive *H. pylori* positive rapid urease test (RUT) or culture. All prescriptions and services in this survey were free of charge. Informed consent was obtained from each patient before the study. This study was approved by our local Ethics Committee.

Sensitivity testing

The E-test was successfully done to demonstrate minimum inhibitory concentrations (MICs) of



Figure 1. Endoscopy Team in Golden Triangle *H. pylori* Community Survey

clarithromycin (CLR), amoxicillin (AMX), ciprofloxacin (CIP), levofloxacin (LVX), metronidazole (MNZ), and tetracycline (TET). The E-test strips of antibiotics were placed on plates and kept for 5-7 days at 37°C, within microaerophilic atmosphere. The MIC was identified by the point of intersection of the halo zone of E-test strip, as previously described (Vilaichone et al., 2016). Strain was considered resistance when the MIC value was >0.12 µg/mL for AMX, >0.5 µg/mL for CLR, >1 µg/mL for levofloxacin LVX, >1 µg/mL for CIP, >8 µg/mL for MNZ and >1 µg/mL for TET. The breakpoints were based upon epidemiological cut-off values (ECOFFs) using EUCAST criteria (2012) (Vilaichone et al., 2013) .

Statistical analysis

The demographic data was obtained using Fisher’s exact test or chi-squared. P-value less than 0.05 was considered as statistically significant. Other data analyses were done by SPSS version 19 (SPSS Inc., Chicago, IL, USA). The study protocol was conducted according to the good clinical practice guidelines and approved by our university ethics committee.

Results

A total of 148 subjects (56 men, 92 women) were included into this study. There were 53 (35.8%) patients infected with *H. pylori* as identified by positive RUT. *H. pylori* infection was demonstrated in 36.3% (37/102) of Chiang khong and 34.8 % (16/46) of Chiang saen subjects. *H. pylori* cultures were successfully performed in 50 patients including 19 men and 31 women with mean age of 55.9 years. The demographic information and details of distribution of all *H. pylori* strains are shown in tTable 1.

Antibiotic susceptibility tests using E-tests for AMX, CLR, MNZ, TET, LVX, and CIP were successfully performed in all culture positive patients (50 strains). Antibiotic resistance was present in 44% (22/50) including CLR (2%), MNZ (26%), CIP (22%), LVX (22%), and multi-drug resistance in 4% of subjects. There was no AMX and TET resistance in this study as shown in Table 2. MNZ resistance was high among Chiang saen and Chiang khong subjects (27.8% and 25% respectively). Chiang khong had higher prevalence of CIP and LVX resistance than Chiang saen patients (25% vs. 16.7%), but none of the differences were statistically significant (Tables 3). Subgroup analysis of each antibiotic and possible

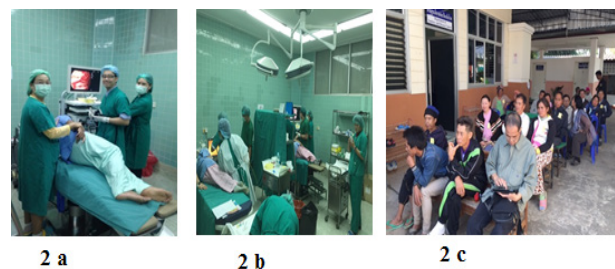


Figure 2. Endoscopy Rooms in Community Hospital at Golden Triangle (2 a, 2 b), Golden Triangle Patients Registering before Undergoing Upper GI Endoscopies (2 c)

Table 1. Demographic Data of All Patients

Demography	Entire cohort (n= 148)	<i>H. pylori</i> positive patients (n=53)	Antibiotic susceptibility tests (n = 50)
Sex			
Men	56	20 (35.7%)	19
Woman	92	33 (35.9%)	31
Mean Age \pm SD (yr)	56.3 \pm 13.3	57 \pm 13.2	55.9 \pm 13.9
Range	21-88	24-85	24-85
< 40 yr.	12	3	4
\geq 40 yr.	136	50	45
Endoscopic finding			
Ulcer	4	2	2
NUD	144	51	48
Locations			
Chiang saen	46	16	18
Chiang khong	102	37	32

Table 2. Prevalence of Antibiotic Resistant *H. Pylori* Strains in Golden Triangle

Antibiotic n = 50	Antibiotic resistance (%)
Amoxicillin (MIC 0.25 μ g/ml)	0
Clarithromycin (MIC 1 μ g/ml)	1(2%)
Metronidazole (MIC 8 μ g/ml)	13 (26%)
Tetracycline (MIC 1 μ g/ml)	0
Ciprofloxacin (MIC 1 μ g/ml)	11 (22%)
Levofloxacin (MIC 1 μ g/ml)	11 (22%)
Multi-drug resistance	2 (4%)

Table 3. Prevalence of Antibiotic Resistant *H. Pylori* Strains in Each Location

Antibiotic (n = 50)	Antibiotic resistance (%)	Chiang saen (n=18)	Chiang klong (n=32)
Amoxicillin (MIC 0.25 μ g/ml)	0	0	0
Clarithromycin (MIC 1 μ g/ml)	0	0	1 (3.1%)
Metronidazole (MIC 8 μ g/ml)	5 (27.8%)	5 (27.8%)	8 (25%)
Tetracycline (MIC 1 μ g/ml)	0	0	0
Ciprofloxacin (MIC 1 μ g/ml)	3 (16.7%)	3 (16.7%)	8 (25%)
Levofloxacin (MIC 1 μ g/ml)	3 (16.7%)	3 (16.7%)	8 (25%)

Table 4. Antibiotic Resistant *H. Pylori* Strains and Clinical Factors in Golden Triangle People

Antibiotics (n = 50)	Sex		Age		Endoscopic findings	
	Female	Male	< 40 years	\geq 40 years	Gastritis (NUD)	PUD
Amoxicillin						
resistant	0.0	0.0	0.0	0.0	0.0	0.0
susceptible	31 (62.0%)	19 (38.0%)	4 (8%)	46 (92.0%)	48 (96.0%)	2 (4.0%)
Clarithromycin						
resistant	0.0	1 (100.0%)	1 (100.0%)	0.0	1 (100.0%)*	0.0
susceptible	30 (61.2%)	19 (38.8%)	4 (8.2%)	45 (91.8%)	47 (91.8%)	2 (8.2%)
Metronidazole						
resistant	7 (53.8%)	6 (46.2%)	1 (7.7%)	12 (92.3%)	13 (100.0%)	0.0
susceptible	24 (64.9%)	13 (35.1%)	3 (8.1%)	34 (91.9%)	35 (94.6%)	2 (5.4%)
Tetracyclin						
resistant	0.0	0.0	0.0	0.0	0.0	0.0
susceptible	31 (62.0%)	19 (38.0%)	4 (8.0%)	46 (92.0%)	48 (96.0%)	2 (4.0%)
Ciprofloxacin						
resistant	8 (72.7%)	3 (27.3%)	0.0	11 (100.0%)	11 (100.0%)	0.0
susceptible	23 (58.9%)	16 (41.1%)	4 (10.3%)	35 (89.7%)	37 (34.8%)	2 (5.2%)
Levofloxacin						
resistant	8 (72.7%)	3 (27.3%)	0.0	11 (100.0%)	11 (100.0%)	0.0
susceptible	23 (58.9%)	16 (41.1%)	4 (10.3%)	35 (89.7%)	37 (34.8%)	2 (5.2%)

*P=0.04

predictive factors (age, sex and endoscopic findings) were performed and demonstrated that prevalence of CLR resistance in gastritis was significantly higher than peptic ulcer patients (100% vs. 0% $P = 0.04$), as depicted in Table 4.

Discussion

Northern region in Thailand have lower economic status compared to Central region of the country and thus would be expected to have a high rate of *H. pylori* infection. There are several previous studies has been reported that *H. pylori* infection in Thailand was decreasing in the past 15 years due to massive *H. pylori* eradication and currently the prevalence of *H. pylori* infection has approximately 35% (Vilaichone et al., 2011, Vilaichone et al., 2013, Srinarong et al., 2014, Prapitpaibool et al., 2015, Vilaichone et al., 2016). In this study, we detected 36.3% of *H. pylori* infection in Golden triangle patients which is comparable to other regions (Vilaichone et al., 2013). Golden triangle used to be opium-producing areas. His majesty the King Rama IX of Thailand has changed Golden triangle area from opium-producing area to value agriculture farms. Our previous king had been striving to seek ways for soil problems through increasing fertility for sandy loam, improving hard setting soil, and finally promoting this area as one of the great resources of temperate fruits producing in Thailand. However, even economic status in this area is developing, many local people still are suffering from upper GI diseases especially *H. pylori* infection. Furthermore, Northern region has highest prevalence of gastric cancer (approximately 10-15: 100,000) in Thailand. Knowing the antibiotic resistant pattern would be very helpful for health care planning to reduce *H. pylori* associated diseases especially gastritis, peptic ulcer diseases, and gastric cancer (Vilaichone et al., 2016).

In line with other studies, we demonstrated that CLR resistance is uncommon in Northern region of Thailand (Vilaichone et al., 2013, Srinarong et al., 2014) which can be due to more popular of new antibiotics such as LVX and moxifloxacin instead of CLR. There was no AMX and TET resistance in Golden triangle compatible with previous report in Northern Thailand (Vilaichone et al., 2016). Fluoroquinolone resistance was present in 25% of Chiang khong district which is the second highest prevalence in Thailand (27.3% in Thai mountain people) (Vilaichone et al., 2016). These findings reflect the frequent use of fluoroquinolone such as ciprofloxacin for GI infections in this area.

Thailand consensus for *H. pylori* treatment in 2015 suggested using sequential or concomitant therapy as the first line regimen (Mahachai et al., 2016) that would be effective regimens in golden triangle due to low CLR resistance. However, the second line treatment with LVX based triple therapy could not be recommended in this part because of high prevalence of fluoroquinolone resistant strain. Rescue therapy after failure of first line treatment in this area should be bismuth-quadruple therapy which consisted of bismuth subsalicylate, proton pump inhibitor (PPI), metronidazole, and tetracycline for 14 days.

Confirmation of eradication from *H. pylori* infection by UBT or stool antigen tests should be performed in all treatment patients to warrant cure of fatal diseases such as gastric cancer, peptic ulcers diseases, and precancerous lesions for gastric cancer (eg. atrophic gastritis, intestinal metaplasia and gastric dysplasia) (Vilaichone et al., 2006).

In conclusion, prevalence of *H. pylori* infection in golden triangle people in Thailand is still high in comparison with other parts of Thailand. MNZ resistance was common; whereas, fluoroquinolone resistance was prevalent and forming a significant problem in this area. Diagnosis of gastritis might be a predictor for CLR resistance in Golden triangle. *H. pylori* eradication with proper regimen using local antibiotic resistant pattern is the key important tool to reduce *H. pylori* associated GI diseases in this particular area.

Acknowledgments

The authors acknowledge the editorial assistance of Ms. Juraiwan Namtanee and Ms. Pornpip Jinpat. This study was partially supported by the Research Fund of the Faculty of Medicine, Thammasat University Hospital, Research grant from Thammasat University and the National Research University Project of Thailand, Office of the Higher Education Commission, Thailand.

References

- Mahachai V, Vilaichone RK, Pittayanon R, et al (2016). Thailand consensus on management of *H. pylori* infection 2015. *Asian Pac J Cancer Prev*, **17**, 2351-60.
- Srinarong C, Siramolpiwat S, Wongcha-um A, et al (2014). Improved eradication rate of standard triple therapy by adding bismuth and probiotic supplement for *Helicobacter pylori* treatment in Thailand. *Asian Pac J Cancer Prev*, **15**, 9909-13.
- Vilaichone RK, Gumnarai P, Ratanachu-Ek T, et al (2013). Nationwide survey of *Helicobacter pylori* antibiotic resistance in Thailand. *Diagn Microbiol Infect Dis*, **77**, 346-9.
- Vilaichone RK, Mahachai V (2001). Current management of *Helicobacter pylori* infection. *J Med Assoc Thai*, **84**, 32-38.
- Vilaichone RK, Mahachai V, Tumwasorn S, et al (2005). Gastric mucosal cytokine levels in relation to host interleukin-1 polymorphisms and *Helicobacter pylori* cagA genotype. *Scand J Gastroenterol*, **40**, 530-39.
- Vilaichone RK, Mahachai V, Graham DY (2006). *Helicobacter pylori* diagnosis and management. *Gastroenterol Clin North Am*, **35**, 229-47.
- Vilaichone RK, Panarat W, Aekpongpaist S, et al (2014). Clinical characteristics and survival of Gastric Cancer in Thailand. *Asian Pac J Cancer Prev*, **15**, 9005-8.
- Vilaichone RK, Prapitpaiboon H, Gamnarai P, et al (2015). Seven-day bismuth-based quadruple therapy as an initial treatment for *Helicobacter pylori* infection in a high metronidazole resistant area. *Asian Pac J Cancer Prev*, **16**, 6089-92.
- Vilaichone RK, Ratanachu-Ek T, Gamnarai P, et al (2016). Extremely high prevalence of metronidazole-resistant *Helicobacter pylori* strains in Mountain people (Karen and Hmong) in Thailand. *Am J Trop Med Hyg*, **94**, 717-20.