Extremely High Prevalence of Metronidazole-Resistant *Helicobacter pylori* Strains in Mountain People (Karen and Hmong) in Thailand

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Abstract. This study aimed to survey the prevalence, patterns of antibiotic resistance, and clinical factors associated with antibiotic resistance in *Helicobacter pylori* among the Karen and Hmong mountain people of Thailand. We recruited dyspeptic patients in the Maesod district, Tak Province, Thailand. All subjects underwent upper gastrointestinal endoscopy, and three antral gastric biopsies were obtained for rapid urease tests and culture. An epsilon meter was used to determine the minimum inhibitory concentrations of amoxicillin (AMX), clarithromycin (CLR), metronidazole (MNZ), levofloxacin (LVX), ciprofloxacin (CIP), and tetracycline (TET). A total of 291 subjects were enrolled; 149 (51.2%) were infected with *H. pylori*. *Helicobacter pylori* infection was present in 47.1% of Thai, 51.7% of Karen, and 58.7% of Hmong subjects. Antibiotic resistance was present in 75.8% including AMX (0.8%), TET (0%), CLR (5.6%), MNZ (71.8%), CIP (19.4%), LVX (19.4%), and multidrug resistance in 21.8%. Karen subjects had the highest prevalence of MNZ resistance (84.6%), and Hmong subjects had the highest prevalence of fluoroquinolone (27.3%) and multidrug (34.1%) resistance. MNZ plus fluoroquinolone (14.5%) was the most common multidrug resistance. There was no association between clinical factors and antibiotic resistance. MNZ resistance was prevalent, whereas fluoroquinolone- and multidrug-resistant *H. pylori* infections are important problems in mountain people of Thailand.

INTRODUCTION

Helicobacter pylori infects almost half of the world's population. *Helicobacter pylori* causes a chronic infection that occurs early in life and is associated with chronic inflammation and long-term consequences such as chronic gastritis, peptic ulcer disease (PUD), mucosa-associated lymphoma, and gastric cancer.^{1,2} The clinical outcomes depend on bacterial virulence, genetic polymorphisms of the host, and environmental factors.^{1,3,4} Mountain people or hill tribes live on both sides of remote border areas between northern Thailand, Laos, and Myanmar. The six major hill tribes within Thailand are the Karen, Hmong, Akha, Lahu, Yao, and Lisu. Each ethnic group has a distinct language and culture. This study involved the Karen and Hmong mountain people of Thailand. The Karen and Hmong people of Thailand usually live in remote villages in the northern and northwestern regions, which are far from towns. The estimated population of the Karen in Thailand is 350,000 people; the majority are Buddhists. Karen people usually live as single families and are primarily involved in agriculture. The Hmong are an ethnic group in the mountain areas of Laos, Vietnam, Myanmar, China, and Thailand. The Hmong are one of the subgroups of the Miao ethnicity in the southern region of China who migrated in the eighteenth century due to political problems. The Hmong are often called mountain people because they mostly live in the high-altitude

areas of Southeast Asian countries. The Hmong are primarily involved in agriculture as their occupation and their culture consists of a dominant male society. The current population of the Hmong in Thailand is estimated to be approximately 150,000 people.

There have not been prior studies of *H. pylori* infection in mountain people of Thailand and Southeast Asian countries. The aim of this study was to survey the prevalence of infection, to assess antibiotic resistance patterns, and to determine if clinical factors are associated with antibiotic resistance in *H. pylori* in the Karen and Hmong people living in rural Thailand.

METHODS

Subjects and sample collection. In November 2013, we conducted a community-based endoscopic survey among dyspeptic patients in the Maesod district, Tak Province (northwestern region), which is 529 km from Bangkok, Thailand (Figures 1 and 2). All participants were villagers living in the same community during our survey. There were three ethnic groups in the community: Thai, Karen, and Hmong. The fraction of each ethnicity was 75% Thai, 15% Karen, and 10% Hmong. The ethnicity was self-described and confirmed by information from community leaders and census registrations. Thai participants lived in the same communities as the Karen and Hmong people. Most of the Karen and Hmong participants were laborers, farmers, or housewives. The majority of Thais were farmers, local traders, or government officers. One month prior to the survey, we contacted community leaders and local hospital officers to announce our project to the community.

Entry criteria included age over 18 years with dyspeptic symptoms and never having received *H. pylori* eradication

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FIGURE 1. Hmong and Karen patients registering before undergoing upper gastrointestinal endoscopies.

therapy or a proton pump inhibitor (PPI), bismuth, or antibiotics in the past month. We excluded those who were receiving anticoagulants, such as warfarin, and those patients with severe systemic diseases, drug, or alcohol abuse. Gastroscopies were performed by gastrointestinal specialists in our team. Three antral gastric biopsies from each patient were obtained and placed into 1.5 mL of transport media. Tubes were kept in a mobile freezer at -20°C and sent to the culture laboratory at Thammasat University Hospital, Pathum Thani, Thailand. The diagnosis of PUD was based on an endoscopic diagnosis. Dyspeptic patients with normal endoscopy and those with gastritis without erosions or ulcers were considered to have gastritis. *Helicobacter pylori* infection was defined as positive *H. pylori* culture or positive rapid urease test (RUT). All *H. pylori* positive patients received standard triple therapy for eradication. All prescriptions and services were free of charge. Informed consent was obtained from all patients, and this protocol was approved by our local ethics committee.

Sensitivity testing. Biopsies were retrieved from the transport media and placed on nonselective media (Columbia agar supplemented with 7% sheep blood; Becton Dickinson, Sparks, MD). Biopsies were spread all over the plate using a

sterile loop, and plates were kept in an microaerophilic incubator (5% O_2 , 10% CO_2 , 85% N_2 , and 95% humidity) at 37°C . The plates were observed daily up for to 10 days and the results were recorded. *Helicobacter pylori* was identified on agar plates by the pattern of colony morphology (small and translucent colonies with a convex elevation). Gram staining was performed to identify Gram-negative rod forms with curved and coccoid forms. The definitive identification of *H. pylori* was based on positive tests for oxidase, catalase, and urease.⁵

The epsilometer test (E-test) was performed to identify the minimum inhibitory concentrations (MICs) of amoxicillin (AMX), clarithromycin (CLR), metronidazole (MNZ), tetracycline (TET), levofloxacin (LVX), and ciprofloxacin (CIP). Viable *H. pylori* colonies were suspended in Mueller Hinton II agar (Becton Dickinson) with 10% horse blood culture media and culture suspension turbidity to three McFarland was applied to inoculate plates. The E-test strip of each antibiotic was placed on a plate and incubated for 5 days at 37°C , under microaerophilic conditions. The MIC was identified by the point of intersection of the inhibition zone with the E-test strip, as previously described.⁵⁻⁸ Strains were considered resistant when the MIC value was $> 0.12 \mu\text{g/mL}$ for AMX, $> 0.5 \mu\text{g/mL}$ for CLR, $> 1 \mu\text{g/mL}$ for LVX, $> 1 \mu\text{g/mL}$ for CIP, $> 8 \mu\text{g/mL}$ for MNZ, and $> 1 \mu\text{g/mL}$ for TET. The breakpoints are based on epidemiological cutoff values, which distinguish wild-type isolates from those with reduced susceptibility by using the European Committee on Antimicrobial Susceptibility Testing criteria (2012).

Statistical analysis. The demographic information was analyzed using the chi-squared test or Fisher's exact test where appropriate. P values < 0.05 were considered to be statistically significant. Data analysis was performed using SPSS version 19 (SPSS Inc., Chicago, IL). The study was conducted according to the good clinical practice guidelines, as well as the Declaration of Helsinki, and was approved by local ethics committee. All participants provided signed informed consent before entry into the study.

RESULTS

A total of 291 subjects were enrolled; 149 (51.2%) were infected with *H. pylori*, as identified by a positive RUT. Cultures were positive in 124 patients including 35 men and 89 women with a mean age of 46.7 years. There were

FIGURE 2. Upper gastrointestinal endoscopies in Hmong and Karen dyspeptic patients.

TABLE 1
Demographic data of all patients

Demography	Entire cohort (N = 291)	<i>Helicobacter pylori</i> positive patients (N = 149)	Antibiotic susceptibility test (E-test) (N = 124)
Sex			
Male	70	44	35
Female	221	105	89
Mean age $\pm$ SD (year)	46.6 $\pm$ 14.4	46.8 $\pm$ 13.8	46.7 $\pm$ 14.6
Range (year)	12–89	12–89	12–89
< 40 years	97	49	44
$\geq$ 40 years	194	100	80
Endoscopic findings			
PUD (14 GU and 4 DU)	18	12 (66.7%)	11
Gastritis	273	137 (50.2%)	113
Ethnicity			
Thai	170	80 (47.1%)	67
Karen	29	15 (51.7%)	13
Hmong	92	54 (58.7%)	44

E-test = epilometer test; GU = gastric ulcer; PUD = peptic ulcer disease; SD = standard deviation; DU = duodenal ulcer.

67 Thai, 44 Hmong, and 13 Karen subjects. The prevalence of infection was 47.1% in Thai subjects, 51.7% in Karen subjects, and 58.7% in Hmong subjects. The E-tests for AMX, CLR, MNZ, TET, LVX, and CIP were successful in all 124 patients. The demographic information and the details of the distribution of all *H. pylori* strains are shown in Table 1.

Antibiotic resistance was present in 75.8% of subjects, including AMX (0.8%), TET (0%), CLR (5.6%), MNZ (71.8%), CIP (19.4%), LVX (19.4%), and multidrug resistance was found in 21.8% of subjects (Table 2). MNZ resistance was high among all three ethnic groups (e.g., > 70%). The Hmong had the highest prevalence of fluoroquinolone (27.3%) and multidrug (34.1%) resistance, but none of the differences were statistically significant. MNZ plus fluoroquinolone (14.5%) was the most common multidrug resistance (Tables 3 and 4). Subgroup analysis between each antibiotic and clinical factors (age, sex, and endoscopic findings) was performed, but there were no significant findings in these analyses (all *P* values > 0.05), as shown in Table 5.

DISCUSSION

The prevalence of *H. pylori* infection in Thailand has been recently estimated as 35%,^{5,9} similar to Laos, a neighboring country that has an *H. pylori* infection rate of 36.2%.¹⁰ Mountain people of Thailand generally have lower economic status with poor sanitation and water supplies compared with other regions of the country and thus would be expected to have a high prevalence of *H. pylori* infection. The preva-

TABLE 2
Prevalence of antibiotic-resistant *Helicobacter pylori* strains in Thai mountain people

Antibiotic (N = 124)	Antibiotic resistance (%)
Amoxicillin	1 (0.8)
Clarithromycin	7 (5.6)
Metronidazole	89 (71.8)
Tetracycline	0
Fluoroquinolone	24 (19.4)
> 1 Drug	27 (21.8)
> 2 Drugs	1 (0.8)

TABLE 3
Prevalence of antibiotic-resistant *Helicobacter pylori* strains in each ethnic group

Antibiotics (N = 124)	Antibiotic resistance (%)		
	Thai (67)	Karen (13)	Hmong (44)
Amoxicillin	0	1 (7.7)	0
Clarithromycin	3 (4.5)	1 (7.7)	3 (6.8)
Metronidazole	47 (70.1)	11 (84.6)	31 (70.5)
Tetracycline	0	0	0
Fluoroquinolone	11 (16.4)	1 (7.7)	12 (27.3)
> 1 Drug	9 (13.4)	2 (15.4)	15 (34.1)
> 2 Drugs	1 (1.5)	1 (7.7)	1 (2.3)

lence of *H. pylori* infection in the mountain people investigated in this study was 51.2%, which was lower than our expectation. However, the prevalence of infection was based on the results of RUT and culture, which may have underestimated. The prevalence of MNZ resistance was high in all three groups and slightly higher than that reported in the southern region of Thailand (66.7%).⁵ Multidrug resistance in the Hmong people was more frequent than in Karen and Thai subjects (34.1%, 15.4%, and 13.4%, respectively). The frequency of multidrug resistance in the Hmong was also greater than that found by the National Thailand study (34% versus 2.7%).⁵ The high prevalence of parasitic and protozoan infections and easy access to over-the-counter antibiotics could likely be an explanation for the high frequency of antibiotic resistance to drugs often used for intestinal infections.

CLR resistance was uncommon in mountain people and has declined in recent years in Thailand. Thailand is now considered to be an area with a low prevalence of CLR resistance.^{5,11} The explanation for this change is likely due to the substitution of newer antibiotics such as LVX and moxifloxacin in place of CLR for respiratory tract infections. The low prevalence of AMX resistance (0.8%) and no resistance to TET (0%) were compatible with previous studies from many countries.^{12–14} Fluoroquinolone resistance was present in 27.3% of Hmong people and possibly reflects the common use of CIP for diarrheal disease in this group.

The efficacy of triple therapy for *H. pylori* eradication has declined worldwide to an unacceptable level in many countries.^{1,3} However, triple therapy using a 14-day high-dose PPI and long-acting CLR has had an excellent cure rate (100%) in Thailand, consistent with Thailand having become a low resistance country.¹¹ Another study from Thailand demonstrated that triple therapy plus bismuth and a probiotic supplement for 14 days yielded 100% *H. pylori* eradication.¹⁵ In Thailand, a 10-day course of sequential therapy or 10 days of concomitant therapy provided a high *H. pylori* eradication

TABLE 4
Multi-drug-resistant *Helicobacter pylori* strains in Thai mountain people

Multidrug resistance (N = 27)	Antibiotic resistance (%)
Metronidazole/amoxicillin	1 (0.8)
Metronidazole/clarithromycin	5 (4)
Metronidazole/fluoroquinolone	18 (14.5)
Metronidazole/fluoroquinolone/clarithromycin	2 (1.6)
Metronidazole/amoxicillin/clarithromycin	1 (0.8)

TABLE 5
Antibiotic-resistant *Helicobacter pylori* strains and clinical factors in Thai mountain people

Antibiotics (N = 124)	Sex		Age		Endoscopic findings	
	Female	Male	< 40 Years	≥ 40 Years	Gastritis	PUD
Amoxicillin						
Resistant	0	1 (100%)	0	1 (100%)	1 (100%)	0
Susceptible	88 (71.5%)	35 (28.5%)	44 (35.8%)	79 (64.2%)	112 (91.1%)	11 (8.9%)
Tetracycline						
Resistant	0	0	0	0	0	0
Susceptible	89 (71.8%)	35 (28.2%)	44 (35.5%)	80 (64.5%)	113 (91.1%)	11 (8.9%)
Clarithromycin						
Resistant	3 (42.9%)	4 (57.1%)	3 (42.9%)	4 (57.1%)	7 (100%)	0
Susceptible	86 (73.5%)	31 (26.5%)	41 (35%)	76 (65%)	106 (90.5%)	11 (9.5%)
Metronidazole						
Resistant	66 (74.2%)	23 (25.8%)	30 (33.7%)	59 (66.3%)	81 (91%)	8 (9%)
Susceptible	23 (65.7%)	12 (34.3%)	14 (40%)	21 (60%)	32 (91.4%)	3 (8.6%)
Fluoroquinolone						
Resistant	18 (75%)	6 (25%)	5 (20.8%)	19 (79.2%)	23 (95.8%)	1 (4.2%)
Susceptible	71 (71%)	29 (29%)	39 (39%)	61 (61%)	90 (90%)	10 (10%)

PUD = peptic ulcer disease.

*Comparison between each antibiotic and age, sex, endoscopic findings were no significant differences (all *P* values > 0.05).

rate (>95%).^{16,17} Both regimens have also been found to be effective in the general Thai population.

In conclusion, the prevalence of *H. pylori* infection in mountain people of Thailand is highly consistent with their socioeconomic status and access to good sanitation and clean water. There have been no prior *H. pylori* treatment studies in this population of mountain people. Thus, future clinical trials for *H. pylori* eradication are needed to indicate the proper regimen for the mountain people of Thailand.

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