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The incidence of primary antibiotic resistance of *Helicobacter pylori* in Vietnam

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Abstract

Goals—To determine the susceptibility of *Helicobacter pylori* strains isolated from a Vietnamese population to 5 antibiotics.

Background—The incidence of antibiotic resistance in *H. pylori* infection is increasing worldwide and has become a leading cause for failure of treatment. Antibiotic susceptibility testing is very important to provide optimal regimens in a clinical setting.

Study—We isolated 103 *H. pylori* strains from the gastric mucosa of *H. pylori*-infected patients from 2 areas in Vietnam (Ho Chi Minh and Hanoi) in 2008. Epsilon test was used to determine the minimum inhibitory concentrations of amoxicillin (AMX), clarithromycin (CLR), metronidazole (MNZ), levofloxacin (LVFX), and tetracycline (TC).

Results—Among the 103 strains, the resistance rates were 0% (AMX), 33% (CLR), 69.9% (MNZ), 18.4% (LVFX), and 5.8% (TC). The resistant strains showed a high-level of resistance (≥256 µg/mL) to CLR, 23.5% (8/34), and MNZ, 29.1% (21/72). The resistance rate for CLR was significantly higher in Ho Chi Minh than in Hanoi (49% vs. 18.5%, $P = 0.001$). Resistance to both CLR and MNZ was most commonly observed (24.3%). Two strains (1.9%) were resistant to 4 of the 5 antibiotics. No significant association was observed between antibiotic resistance rates and age, gender, or clinical outcomes of the patients.

Conclusions—High incidence of resistance to CLR and MNZ suggests that standard triple therapies may not be useful as first-line treatment in Vietnam. Alternative strategies such as bismuth-based quadruple therapies or sequential therapy may be more effective in Vietnam.

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Keywords

Helicobacter pylori, drug resistance; Vietnam

Introduction

Helicobacter pylori is a spiral Gram-negative bacterium that infects more than half of the world's population and is currently thought to be involved in the pathogenesis of chronic gastritis, peptic ulcer diseases, gastric cancer, and mucosa-associated lymphoid tissue (MALT) lymphoma^{1, 2}. Eradication of *H. pylori* not only heals peptic ulcers but also prevents their recurrence and reduces the risk of development of gastric cancer^{3–7}. Furthermore, other *H. pylori*-associated disorders such as MALT lymphoma, atrophic gastritis, and intestinal metaplasia regress after treatment with antibiotics^{8–10}.

Triple therapy regimens including 1 proton pump inhibitor (PPI) and 2 antimicrobial agents such as amoxicillin (AMX), clarithromycin (CLR), metronidazole (MNZ), levofloxacin (LVFX), and tetracycline (TC) have been widely used to eradicate this bacterium. Although the success of the treatment depends on several factors such as smoking and patient compliance, antibiotic resistance is the most common factor causing failure of treatment^{11–13}. The incidence of antibiotic resistance is increasing worldwide and varies according to the geographic location; antibiotic resistance is generally higher in developing countries than in developed regions^{14–16}. In addition, the rate of antibiotic resistance often correlates with the amount of antibiotics consumed in the population^{14, 17–19}. In Vietnam, no domestic guidelines are available for the treatment of *H. pylori* because of insufficient domestic data. Therefore, CLR- and/or MNZ-based triple therapy is commonly used as the standard primary regimen for the treatment of *H. pylori* infection on the basis of the international consensus reports^{7, 20, 21}. Although information about the antimicrobial susceptibility pattern is important for improving the outcome of the primary eradication therapy, it is difficult to perform these antimicrobial susceptibility tests in a clinical setting in developing countries such as Vietnam. Therefore, an eradication regimen based on an empirical approach is used without an initial susceptibility testing in Vietnam. Although a previous study has reported the rates of antibiotic resistance in Vietnam, this study was performed only on subjects recruited from Hanoi between 1999 and 2001²². In their study, the rates of resistance to CLR and MNZ were 1% and 76%, respectively²². Because the rate of resistance to CLR is increasing throughout the world^{23–25}, it is necessary to examine the recent drug resistance rates in Vietnam. In this study, we aimed to determine the antibiotic susceptibility of *H. pylori* to AMX, CLR, MNZ, LVFX, and TC using strains isolated from the Vietnamese populations living in northern (Hanoi) and southern regions (Ho Chi Minh).

Materials and Methods

Patients and *H. pylori*

H. pylori strains were obtained as described in our previous study from the gastric mucosa of *H. pylori*-infected Vietnamese patients who underwent endoscopy at Cho Ray Hospital in Ho Chi Minh and Bach Mai Hospital in Hanoi in 2008²⁶. Peptic ulcer disease, including gastric ulcer and duodenal ulcer, was diagnosed by endoscopic observation, while chronic gastritis was determined by histological examination. Exclusion criteria included a history of partial gastric resection, eradication therapy for *H. pylori*, treatment with bismuth-containing compounds, H₂-receptor blockers, or proton pump inhibitors within 4 weeks before the study. Informed consent was obtained from all participants, and the protocol was approved by the Ethics Committee of Cho Ray Hospital and Bach Mai Hospital.

For *H. pylori* culture, 2 biopsy samples (one from the antrum and one from the corpus) were homogenized in saline and inoculated onto Mueller–Hinton Agar medium (Becton Dickinson, NJ, USA) supplemented with 7% defibrinated horse blood (Horse blood, Biotest, Japan) without antibiotics. The plates were incubated for up to 10 days at 37°C under microaerophilic conditions (10% O₂, 5% CO₂, and 85% N₂) in an incubator (MCO-5M [UV]; Maruchi Incubator, SANYO, Japan). We selected several small round colonies from each patient's plate and subcultured them 1 or 2 times to obtain a pure culture. *H. pylori* was identified on the basis of colony morphology, Gram staining, and positive reaction for oxidase, catalase, and urease. The isolated strains were stored at –80°C in Brucella Broth (Dico, NJ, USA) containing 10% dimethylsulfoxide and 10% horse serum.

Antibiotic susceptibility testing

Epsilometer test (E-test) (AB Biodisk, Sweden) was used to determine the minimum inhibitory concentrations (MICs) of AMX, CLR, MNZ, LVFX, and TC. Mueller–Hinton II Agar medium (Becton Dickinson) supplemented with 10% defibrinated horse blood was used as culture media, and the culture suspension with turbidity adjusted to be equivalent to a McFarland opacity standard of 2.0 to 3.0 was used to inoculate onto the plates. The E-test strip of the corresponding antibiotic was placed on the plate and incubated for 3 to 5 days at 37°C under microaerophilic conditions. MIC was defined as the point of intersection of the elliptical inhibition zone with the E-test strip. Strains were considered as resistant when the MIC was >1 µg/mL for AMX, 1 µg/mL for CLR, 1 µg/mL for LVFX, 8 µg/mL for MNZ, and 4 µg/mL for TC²⁷. To confirm the results, each test was performed at least 3 times using new corresponding E-test strips.

Statistical analysis

All statistical analyses were performed by SPSS version 19 (SPSS Inc., Chicago, IL, USA). The average age was compared using the unpaired *t*-test or Mann–Whitney *U*-test according to parametric or nonparametric distribution. The univariate association between each factor was quantified using Fisher's exact test and chi-square test. Differences in the incidence of CLR resistance in 2 areas were analyzed using adjusted by gender using the Mantel–Haenszel method. A two-tailed *P* value of <0.05 was considered as statistically significant.

Results

We isolated 103 strains of *H. pylori* that were confirmed by culture from *H. pylori*-positive Vietnamese patients (49 from Ho Chi Minh and 54 from Hanoi; 47 men; age range, 19–83 years; mean age, 47.3 ± 13.7 years and 56 women; age range, 14–66 years; mean age 42.3 ± 9.6 years). Among these *H. pylori*-positive patients, 78 had chronic gastritis and 25 had peptic ulcer diseases including gastric ulcer (12) and duodenal ulcer (13). *H. pylori* showed the highest rate of resistance to MNZ (72/103, 69.9%) (Table 1). The rates of resistance to CLR, LVFX, and TC were 34/103 (33.0%), 19/103 (18.4%), and 6/103 (5.8%), respectively. On the other hand, none of the strains in this study was resistant to AMX. Eighteen strains were susceptible to all tested antibiotics. Although the rate of resistance to CLR was higher in patients with gastric ulcer than with of duodenal ulcer, the value was not statistically significant (*P* = 0.07). There were no differences in the rates of resistance to MNZ, LVFX, and TC between patients with gastric ulcer and duodenal ulcer. The distribution of age and antimicrobial resistance is shown in Table 2. Antibiotic resistance did not differ among different age groups (*P* = 0.76, 0.12, 0.17, and 0.59 for CLR, MNZ, LVFX, and TC, respectively). Similarly, no significant association was observed between the rate of antibiotic resistance and the gender of the patients (data not shown). The distribution of MIC values for each antibiotic is shown in Table 3. *H. pylori* showed a high level of resistance (>256 µg/mL) to CLR, 23.5% (8/34), and MNZ, 29.1% (21/72).

We compared the resistance rates between the 2 areas (Ho Chi Minh and Hanoi). The resistant rate for CLR was significantly higher in Ho Chi Minh than in Hanoi (49.0% vs 18.5%, $P = 0.001$; Table 4). Despite adjustment of the samples for gender using the Mantel–Haenszel method, the resistance rate for CLR was significantly higher in Ho Chi Minh than in Hanoi ($P = 0.001$). On the other hand, the resistance rates of MNZ, LVFX, and TC, among the 4 remaining antibiotics, were not different between Ho Chi Minh and Hanoi.

The distribution of the multidrug resistant strains is shown in Table 5. Of all the strains, 24.3% (25/103) showed double-drug resistance to CLR and MNZ. Resistance to 3 antibiotics CLR, MNZ, and LVFX was observed in 8 (7.8%) strains. Two (1.9%) strains were resistant to 4 out of the 5 antibiotics. No differences were observed in clinical outcomes between single drug resistance and multidrug resistance ($P = 0.81$).

Discussion

The incidence of *H. pylori* infection differs widely between developed and developing countries. In developing countries such as India and Saudi Arabia and African countries, the infection rate is high and approximately 80% of the population infected with *H. pylori* by 20 years old²⁸. In contrast, the infection rate is reported to be as low as 10% to 20% in developed countries, and the infection rate increases at a rate of approximately 1% per year²⁹. In Japan, the incidence of *H. pylori* infection in patients under the age of 10 years is very low (eg, approximately 5%), and the incidence of infection increases with age³⁰. The state of environmental hygiene is related to the *H. pylori* infection rate. In Vietnam, the incidence of *H. pylori* infection increases with age, and it is more than 70% in 30-year-old patients³¹.

Vietnam can be categorized as an area with an intermediate risk of gastric cancer³²; therefore, successful eradication therapy is important to reduce the risk of developing gastric cancer. However, unfortunately, we found that the antibiotic resistant strains were common in the Vietnamese population. Our present study showed that the resistance rate for CLR and MNZ, which are recommended as first-line therapies in Asian countries²⁰, was 33% and 69.9%, respectively. Furthermore, over 20% of resistant strains showed high-level antibiotic resistance. Although the patient's lack of compliance, inadequate length of therapy, or a high bacterial burden are conditions that may contribute to a loss of efficacy, antimicrobial resistance is regarded as the leading factor responsible for failure of eradication therapy. This issue is of particular relevance with regard to CLR, which can induce a virtually 70% loss of effectiveness depending on macrolide susceptibility *in vitro*¹⁴. In addition, meta-analysis showed that triple therapy consisting of PPI, AMX, and CLR in CLR resistance decreased treatment efficacy by 66%³³. In fact, the Maastricht III guidelines on *H. pylori* infection management recommend substituting MNZ for CLR when resistance to this antibiotic exceeds 15% to 20%⁷.

However, unfortunately, the resistance rate for MNZ was also very high in Vietnam. The high incidence of MNZ-resistant strains observed in this study might be attributed to the widespread over-the-counter (OTC) use of this drug. MNZ is used frequently to treat not only *H. pylori* infection but also other infections such as intestinal parasites, periodontal, and gynecological diseases, which are common in developing countries, including Vietnam^{14, 34}. Recently, the resistance rate for MNZ has increased, and this is a main factor leading to the reduction in the efficacy of standard triple therapy in most countries²⁰. Although *in vitro* resistance to MNZ may not accurately reflect *in vivo* resistance, regimens including MNZ are not a preferable choice in populations with >40% MNZ resistance^{17, 35}. Therefore, MNZ will not be useful as a first-line regimen instead of CLR in Vietnam. Interestingly, the resistance rate for CLR was significantly higher in Ho Chi Minh (49.0%)

than in Hanoi (18.5%) in this study. Although information on the use of antibiotics has not been fully reported in Vietnam, this finding may in part be attributed to the different usage of this antibiotic between the 2 areas. These results suggest that CLR can still be useful in Hanoi but not in Ho Chi Minh. However, we should keep in mind that CLR resistance rate of our strains was higher than the strains isolated in 1999 to 2001 from Hanoi (18.5% vs 1.9%, respectively)²². Further surveillance for CLR resistance rate is required.

Recently, LVFX has been prescribed as a rescue drug to eradicate infection in patients with failure of first-line therapy^{36, 37}. However, the incidence of LVFX resistance seems to be increasing worldwide, and this may reduce the efficacy of treatment with LVFX-based regimens^{38–43}. Therefore, according to European, Asia-Pacific, and American guidelines, LVFX should be used in salvage therapy based on antibiotic susceptibility testing^{7, 20, 21}. In Vietnam, LVFX is rarely used for the treatment of other infectious diseases. However, we observed a relatively high resistance rate of LVFX (18.5%) which is a cause of concern. Other fluoroquinolones such as nalidixic acid, ciprofloxacin, and ofloxacin, which are commonly used in Vietnam, may lead to cross resistance with LVFX. On the other hand, TC resistance was rare (5.8%), which is consistent with that reported in previous studies from other countries^{44–46}. TC is not often used for infectious diseases in Vietnam. Therefore, TC-based or quadruple therapy including TC can be a useful alternative first-line regimen in Vietnam as recommended in guidelines^{7, 20}. Resistance to AMX is absent or very low and varies in different countries^{45, 47, 48}. Similar to the findings reported in most studies, our results showed that all strains were susceptible to AMX, which was consistent with a previous study in Hanoi²². However, because AMX is one of the most commonly used antibiotics in Vietnam, no resistant strain observed would be questioned. Indeed, AMX resistance develops by genomic mutation in the *pbp1A* gene,^{27, 49, 50} and resistant phenotype may be lost after storage or freezing^{51, 52}.

Multidrug resistance has recently appeared as a serious challenge in the fight against infections in over the world. *H. pylori* strains harboring triple or quadruple resistance can hinder the choice and success of the eradication regimen. Our finding showed that 33% (34 strains) of the isolates were the resistant to at least 2 antibiotics. Resistance to CLR and MNZ was most commonly observed (24.3%), and this may be the main reason for failure of treatment in Vietnam. Interestingly, we observed that 2 strains (1.9%) were resistant to 4 out of the 5 antibiotics used in this study. Quadruple resistance has been reported only in a few studies in countries such as India (1.7%) and Bulgaria (0.7%)^{53, 54}. The presence of multidrug resistance in Vietnam may be attributed to the increasing use of antibiotics across the country; however, sufficient information about the overall increase in antibiotic use in Vietnam is not available. It is important to perform a susceptibility-guided retreatment using a case-by-case approach, if available, in patients with failure of initial treatment. Other alternative strategies such as bismuth-based quadruple therapies or sequential therapy may be more effective in Vietnam^{7, 20, 55}. To date, the effectiveness of these therapies has not been established. Further clinical trials are required to improve the rate of successful eradication in Vietnam.

In conclusion, the rates of resistance to CLR and/or MNZ were high in Vietnam, which suggests that CLR-based or MNZ-based triple therapy cannot be useful as first-line therapies in Vietnam. TC can be still used although the domestic data for the successful eradication rate is lacking. National epidemiological surveillance of resistance rates is required to select the optimal treatment strategies in Vietnam.

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Figure 1.
Distribution of antibiotic resistance in Ho Chi Minh and Hanoi

Table 1

Antibiotic susceptibility of 103 *H. pylori* strains isolated in Vietnam

Antibiotics	No. (%) of resistance isolates in patients				
	All patients (n=103)	PUD (n=25)	CG (n=78)	Male (n=47)	Female (n=56)
AMX	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
CLR	34 (33.0)	10 (40)	24 (30.8)	13 (27.6)	21 (44.7)
MNZ	72 (69.9)	19 (76)	53 (67.9)	31 (65.9)	41 (73.2)
LVFX	19 (18.4)	5 (20)	14 (17.9)	6 (12.8)	13 (23.2)
TC	6 (5.8)	2 (8)	4 (5.1)	2 (4.2)	4 (7.1)

PUD; Peptic Ulcer Disease, CG; Chronic Gastritis

AMX; amoxicillin, CLR; clarithromycin, MNZ; metronidazole, LVFX; levofloxacin, TC; tetracycline

Table 2

Age distribution of antibiotic resistance in Vietnam

	Age group					Total
	14 – 34 y n (%)	35 – 49 y n (%)	50 – 64 y n (%)	65 n (%)		
Total	20	50	24	9	103	
CLR	5 (25.0)	19 (38.0)	7 (29.2)	4 (44.4)	34	
MNZ	13 (65.0)	36 (72.0)	14 (58.3)	9 (100.0)	72	
LVFX	3 (15.0)	7 (14.0)	5 (20.8)	4 (44.4)	19	
TC	2 (10.0)	2 (4.0)	1 (4.2)	1 (11.1)	6	

CLR; clarithromycin, MNZ; metronidazole, LVFX; levofloxacin, TC; tetracycline

n; number of each antibiotics in each age group, y; years

The number presenting in parentheses shows the percentage of resistant rate for each antibiotic in each age group

Table 3

Distribution of MIC ($\mu\text{g/ml}$) in *H. pylori* isolates in Vietnam

MIC	1	1 – < 64	64 – < 128	128 – < 256	256
AMX (%)	103 (100)	0 (0)	0 (0)	0 (0)	0 (0)
MIC	< 1	1 – < 64	64 – < 128	128 – < 256	256
CLR (%)	69 (67)	21 (20.4)	3 (2.9)	2 (1.9)	8 (7.8)
MIC	< 8	8 – < 64	64 – < 128	128 – < 256	256
MNZ (%)	31 (30.1)	39 (37.9)	9 (8.7)	3 (2.9)	21 (20.4)
MIC	< 1	1 – < 32	32	NA	NA
LVFX (%)	84 (81.6)	5 (4.8)	14 (13.6)		
MIC	< 4	4 – < 64	64 – < 128	128 – < 256	256
TC (%)	97 (94.2)	5 (4.9)	0 (0)	0 (0)	1 (0.9)

NA: not available, MIC reading scale of LVFX ranges from 0.002 to 32 $\mu\text{g/ml}$

AMX; amoxicillin, CLR; clarithromycin, MNZ; metronidazole, LVFX; levofloxacin, TC; tetracycline

The number presenting in parentheses shows the percentage in each antibiotic group

Table 4

Distribution of antibiotic resistance in Ho Chi Minh and Hanoi

	Ho Chi Minh	Hanoi	p-value
n	49	54	
Age	45.0±13.2	44.2±12.9	0.78
Gender:			
Male	13 (26.5%)	34 (63.0%)	<0.001
Diseases			
PUD	9 (18.4%)	16 (29.6%)	0.18
CG	40 (81.6%)	38 (70.4%)	0.365
CLR	21 (49.0%)	10 (18.5%)	0.001
MNZ	34 (69.3%)	38 (70.3%)	0.91
LVFX	9 (18.3%)	10 (18.5%)	0.98
TC	2 (4.0%)	4 (7.4%)	0.44

PUD; Peptic Ulcer Disease, CG; Chronic Gastritis

CLR; clarithromycin, MNZ; metronidazole, LVFX; levofloxacin, TC; tetracycline

Table 5Multidrug resistance patterns of *H. pylori* in Vietnam

Resistance patterns	Number	Percentage
Double drugs		
CLR+MNZ	25	24.3%
MNZ+LVFX	16	15.5%
CLR+LVFX	9	8.7%
CLR+TC	3	2.9%
MNZ+TC	3	2.9%
LVFX+TC	3	2.9%
Triple drugs		
CLR+MNZ+LVFX	8	7.8%
CLR+MNZ+TC	3	2.9%
CLR+LVFX+TC	2	1.9%
MNZ+LVFX+TC	2	1.9%
Quadruple drugs		
CLR+MNZ+LVFX+TC	2	1.9%

CLR; clarithromycin, MNZ; metronidazole, LVFX; levofloxacin, TC; tetracycline