Pattern of Drug Resistant *Helicobacter Pylori* in Dyspeptic Patients in Thailand

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Abstract

Emergence of drug resistant *Helicobacter pylori (H. pylori)* has occurred in various countries and could compromise the efficacy of current treatment regimens. The aim of the study was to identify the pattern of antibiotic resistant *H. pylori* in Thailand and evaluate various factors associated with drug resistance. Between June 2001 and December 2002, a total of 560 dyspeptic patients who underwent upper gastrointestinal endoscopy at King Chulalongkorn Memorial Hospital were included in this study. Antral gastric biopsies were obtained for *H. pylori* cultures and susceptibility tests using Epsilometer test (E-test). The value of antibiotic resistant breakpoints were amoxicillin 0.5 μg/ml, clarithromycin 1.0 μg/ml, metronidazole 8 μg/ml, and tetracycline 4 μg/ml, respectively. *H. pylori* were detected in 315 patients using the rapid urease test (56.25%). Cultures for *H. pylori* were positive in 172 patients. E-test for all four antibiotics was successfully placed in 79 isolations. The prevalence of antibiotic resistant *H. pylori* were amoxicillin 13.9 per cent (11/79), clarithromycin 19.0 per cent (15/79), metronidazole 30.4 per cent (24/79), tetracycline 5.1 per cent (4/79), and multi-drugs 16.5 per cent (13/79), respectively. However, age, sex, or endoscopic findings did not differ between the patients with *H. pylori* resistant strains and sensitive strains. The emergence of antibiotic and multi-drug resistant *H. pylori* in Thailand were relatively high and these could compromise the efficacy of current treatment regimens. The factors associated with drug resistant *H. pylori* could not be demonstrated in the present study. Further study in a larger number of patients might be necessary to identify factors associated with resistant *H. pylori.*

Key word : *Helicobacter Pylori*, Drug Resistance, Dyspeptic Patients, Thailand

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Helicobacter pylori (*H. pylori*) is a major causative agent for chronic and peptic ulcer diseases (1,2). Furthermore, *H. pylori* plays a pivotal role in the pathogenesis of gastric cancer and gastric mucosa-associated lymphoid tissue (MALT) lymphoma(3,4). Eradication of *H. pylori* has been shown to improve the outcome of peptic ulcer diseases in-terms of reducing recurrence and complications. Antibiotic treatment of *H. pylori* infection is complex, requiring multiple antibiotics with acid suppressive agents to achieve high cure rates. Treatment failure is attributed generally to the lack of compliance with the regimen or to antibiotic resistance of the organism(5,6). Emergence of resistant *H. pylori* has been documented in various countries and threatens to compromise current treatment regimens(7-11). However, data on resistance are generally obtained from unique subset of populations over a short period. The studies have shown that demographic factors play an important role in the reported rates of antibiotic resistance in *H. pylori*. Metronidazole resistance is higher in developing countries and especially high in women(12-14). A previous study reported that clarithromycin resistance was significantly associated with older age, women gender, and ulcer status(15,16). The aim of the study was to identify antibiotic resistant *H. pylori* in Thailand and evaluate factors associated with drug resistance.

MATERIAL AND METHOD

A total of 560 dyspeptic patients who underwent upper gastrointestinal endoscopy at King Chulalongkorn Memorial Hospital between June 2001 and December 2002 were included in this study. Informed consent was obtained from each patient. The exclusion criteria included: 1) previous use of proton pump inhibitors (PPIs) or antibiotics within 1 month before the endoscopy, 2) previous history of *H. pylori* eradication, 3) bleeding tendency.

Three gastric biopsy specimens were obtained from the antrum of each patient. One piece for rapid urease test and the other two pieces for culture and susceptibility test. Biopsies were transported in sterile screw-capped tubes containing cysteine medium. Then specimens from each patient were inoculated in two plates. One was non-selective horse blood agar (HBA) plate and the other was selective horse blood agar plate that contained vancomycin and amphotericin. Plates are incubated for 72 hours in microaerophilic condition, at 37°C. Plates were read and the results recorded daily for up to 14 days. The bacterial growth resulting from the primary culture plates was indentified as *H. pylori* by colony morphology and Gram's stain reaction, and by catalase, urease and oxidase reactions. Isolates from each positive plate were transferred to a fresh HBA plate, then incubated for 72 hours. All stock cultures were placed in each labelled vial and stored at -70°C. Susceptibility tests to amoxicillin, clarithromycin, metronidazole, and tetracycline were determined using the Epsilometer test (E-test ; AB Biodisk, Solna, Sweden). *H. pylori* isolates were suspended in Columbia broth to achieve a McFarland opacity of 2 and spread on HBA plates. The antimicrobial drug strip was placed on the plate an incubated for 72 hours. The minimum inhibitory concentration (MIC) was defined by the point of intersection of the inhibitory zone with the strip. Drug resistance was considered when the MIC value was greater than 0.5 μg/ml for amoxicillin, greater than 1 μg/ml for clarithromycin, greater than 8 μg/ml for metronidazole, and greater than 4 μg/ml for tetracycline, respectively.

Data were analyzed with SPSS program version 10.0. The association between sex, age, and endoscopic findings and drug resistant *H. pylori* was evaluated by the two tailed Chi-square and Fisher's exact tests. The significance was set at a p-value less than or equal to 0.05.

RESULTS

From the 560 dyspeptic patients enrolled in this study, *H. pylori* was detected by positive rapid urease test in 315 cases (56.25%). Cultures for *H. pylori* were achieved in 172 patients (54.6%), and E-tests for all four antibiotics were successfully placed in 79 isolations. There were 44 men and 35 women, mean age 51.5 years (range 21-87 years). Endoscopic findings were normal in 7 patients (8.9%), gastritis in 38 patients (48.1%), gastric ulcer in 22 patients (27.8%), duodenal ulcer in 9 patients (11.4%) and both gastric and duodenal ulcer in 3 patients (3.8%). (Table 1)

The antibiotic resistance to amoxicillin, clarithromycin, metronidazole and tetracycline was found in 13.9 per cent, 19.0 per cent, 30.4 per cent and 5.1 per cent of *H. pylori* isolates, respectively. The multidrugs resistance was revealed in 13 patients (16.5%). The susceptibilities of the four antibiotics are listed in Table 2.

Table 3 shows the resistance to each of the four antibiotics by sex, age group and endoscopic

Table 1. Characteristics of the study population.

	Number	Per cent
Sex		
Men	44	55.7
Women	35	44.3
Age		
< 60 yrs	54	68.4
≥ 60 yrs	25	31.6
Endoscopic findings		
Normal	7	8.9
Gastritis	38	48.1
Gastric ulcer	22	27.8
Duodenal ulcer	9	11.4
Both gastric and duodenal ulcer	3	3.8

findings. There was no statistical difference in the rate of resistance to any of the four antibiotics tested when compared between gender, age groups (age < 60 years *versus* ≥ 60 years), or endoscopic findings ($p > 0.05$).

DISCUSSION

Because *H. pylori* is difficult to grow and stock for complete sensitivity testing, in the present study E-test for all 4 antibiotics was successfully placed in only 45.9 per cent (79/172) of the patients whose culture was positive.

In the present study amoxicillin resistance rate was higher than in a previous study (1.4%)[17]. The difference may be partly from the difference of geographic region. E-test MIC value for amoxicillin has been reported to be within one to two fold of MIC values on agar dilution. Confirmation of amoxicillin resistance *in vitro* by agar dilution may be necessary. Furthermore, the effect of the *in vitro* amoxicillin resistance on the outcome of eradication of *H. pylori* should be studied. In the present study clarithromycin resistance rate was 19.0 per cent. In most developed countries the prevalence of primary resistance to clarithromycin appears to be low, less than 10 per cent [18]. Prevalence of clarithromycin resistance higher than 10 per cent has been reported in Belgium, France, Portugal, Peru, Poland, Hongkong, and Japan[19-24]. As discussed above, clarithromycin resistance may influence the eradication rate. In the present study amoxicillin resistance was significantly high so it may cause a significant decrease in the eradication rate with the amoxicillin-clarithromycin containing regimen. In the future, quadruple regimens (a proton-pump inhibitor, bismuth, tetracycline and metronidazole) may be the treatment of choice in the emergence of multi-drug resistant *H. pylori* infection.

In the present study there was no difference in the rate of resistance to any of four antibiotics when comparing age, sex or endoscopic findings. This result may be due to the insufficient number of isolates studied for *H. pylori* resistance. A larger number

Table 2. *In vitro* susceptibility of *H. pylori* isolates (n = 79).

Antibiotics	Number of resistant patients	%
Amoxicillin	11	13.9
Clarithromycin	15	19.0
Metronidazole	24	30.4
Tetracycline	4	5.1
Amoxicillin + clarithromycin	5	6.3
Amoxicillin + metronidazole	5	6.3
Amoxicillin + tetracycline	4	5.1
Clarithromycin + metronidazole	7	8.9
Clarithromycin + tetracycline	2	2.5
Metronidazole + tetracycline	1	1.3
Amoxicillin + clarithromycin + metronidazole	3	3.8
Amoxicillin + metronidazole + tetracycline	1	1.3
Amoxicillin + clarithromycin + tetracycline	2	2.5
Clarithromycin + metronidazole + tetracycline	1	1.3
≥ 1 drug	36	45.6
≥ 2 drugs	13	16.5
≥ 3 drugs	4	5.1
All 4 drugs	1	1.3

Table 3. Antimicrobial resistance of four drugs by sex, age, and endoscopic findings.

Antibiotics	Sex		Age		Endoscopic findings	
	Female	Male	> 60 yrs	≥ 60 yrs	No ulcer	Ulcer detected
Amoxicillin						
Resistant	6	5	5	6	4	7
Susceptible	29	39	20	48	41	27
Clarithromycin						
Resistant	8	7	6	9	7	8
Susceptible	27	37	19	45	38	26
Metronidazole						
Resistant	10	14	10	14	11	13
Susceptible	25	30	15	40	34	21
Tetracycline						
Resistant	2	2	1	3	3	1
Susceptible	33	42	24	51	42	33
Multi-drug resistant						
Yes	7	6	7	6	6	7
No	37	29	47	19	39	27

of *H. pylori* strains may be required to identify risk factors that are associated or predict the antimicrobial resistance pattern.

Culture and sensitivity test for *H. pylori* is not recommended in routine practice. In conclusion, the authors reported a high rate of resistance to amoxicillin and clarithromycin in *H. pylori* isolates in Thailand. Pre-treatment resistance to amoxicillin and clarithromycin may have a significant negative impact on treatment outcome. Susceptibility information on *H. pylori* should continue to be collected to allow researchers to follow trends in antimicrobial resistance and better evaluate the significance of the risk factors.

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ภาวะดื้อยาของเชื้อ*เฮลิโคแบคเตอร์ ไพโลไร* ในผู้ป่วยที่มาด้วยอาการปวดท้องส่วนบนในประเทศไทย

ณัฐญา ตั้งมั่นคงวรกูล, พบ, วโรชา มหาชัย, พบ*, ดวงพร ทองงาม, พบ**, รัฐกร วิไลชนม์, พบ*, สมหญิง ธัมวาสร, ดร***, พินิจ กุลละวณิชย์, พบ**

มีรายงานจากหลายประเทศ เกี่ยวกับภาวะการดื้อยาหลายชนิดของเชื้อ*เฮลิโคแบคเตอร์ ไพโลไร* ซึ่งจะมีผลกระทบต่อการรักษา วัตถุประสงค์ของการศึกษาเพื่อที่จะดูภาวะการดื้อยาหลายชนิดของ เชื้อ*เฮลิโคแบคเตอร์ ไพโลไร* ในประเทศไทย และประเมินปัจจัยที่เกี่ยวข้องกับการดื้อยาระยะเวลาที่ทำการศึกษา ระหว่างมิถุนายน พ.ศ. 2544 ถึง ธันวาคม พ.ศ. 2545 ผู้ป่วยจำนวน 560 คน ที่มาด้วยอาการปวดท้องและเข้ารับการตรวจวินิจฉัยด้วยการส่องกล้องระบบทางเดินอาหารส่วนบนที่โรงพยาบาลจุฬาลงกรณ์ ได้รับคัดเลือกเข้าร่วมในการศึกษานี้ การเพาะเชื้อและทดสอบความไวของเชื้อต่อยาปฏิชีวนะ โดยใช้ Epsilometer test (E-test) โดยระดับที่ถือว่าดื้อยาคือ amoxicillin > 0.5 μg/ml, clarithromycin > 1.0 μg/ml, metronidazole > 8 μg/ml และ tetracycline > 4 μg/ml ตรวจพบ เชื้อ*เฮลิโคแบคเตอร์ ไพโลไร* จากวิธี rapid urease test ให้ผลบวก 315 ราย (56.25%) สามารถเพาะเชื้อขึ้น 172 ราย หลังจากนั้นนำไปวาง E-test สำหรับยาปฏิชีวนะได้ครบทั้ง 4 ชนิด 79 รายผลการศึกษาพบว่าความชุกของการดื้อยาเป็นดังนี้ amoxicillin 13.9% (11/79), clarithromycin 19.0% (15/79), metronidazole 30.4% (24/79), tetracycline 5.1% (4/79), และดื้อยา 2 ชนิดขึ้นไป 16.5% (13/79) อย่างไรก็ตามไม่พบว่ากลุ่มอายุ เพศ หรือ ลักษณะที่พบจากการส่องกล้องมีความสัมพันธ์กับการดื้อยาของเชื้อ*เฮลิโค แบคเตอร์ ไพโลไร* ($p > 0.05$)

โดยสรุป อัตราการดื้อยาปฏิชีวนะของเชื้อ*เฮลิโคแบคเตอร์ ไพโลไร* ในประเทศไทยค่อนข้างสูง และอาจมีผลต่อการรักษาเพื่อกำจัดเชื้อชนิดนี้ อย่างไรก็ตามไม่สามารถหาปัจจัยที่มีความเกี่ยวข้องต่อการดื้อยาได้ ดังนั้นจึงควรจะศึกษาต่อไปเพื่อเก็บข้อมูลให้มากกว่านี้ ในการยืนยันผลที่ได้จากการศึกษา

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