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Short communication

Prevalence of antimicrobial resistance in *Helicobacter pylori* isolates in Taiwan in relation to consumption of antimicrobial agents

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ABSTRACT

During 1998–2004, a total of 218 *Helicobacter pylori* isolates were obtained from patients who were randomised to receive one of the following regimens in a medical centre in Taiwan: lansoprazole, amoxicillin and clarithromycin (LAC) therapy; or lansoprazole, metronidazole and clarithromycin (LMC) therapy. In the LMC group, resistance rates for metronidazole and clarithromycin reduced from 48.6% (1998–2000) to 20.4% (2001–2004) ($P < 0.05$) and from 13.5% to 6.3% ($P < 0.05$), respectively. Analysis of annual antimicrobial consumption found that metronidazole use was slowly decreased both in the total population and in gastrointestinal disease patients. The per-protocol analysis revealed a higher eradication rate for patients using LMC therapy in 2001–2004 (82.6% vs. 75.0%), whilst there was similar efficacy for LAC therapy (84.8% vs. 84.2%). This observation suggests an effective programme to control *H. pylori* antibiotic resistance and hence elevate its cure rate.

1. Introduction

Helicobacter pylori is a major aetiological agent for chronic gastritis, which may lead to more severe disorders including gastric ulcer, duodenal ulcer and gastric adenocarcinoma [1]. Eradication of *H. pylori* improves ulcer healing and reduces the recurrence of gastric and duodenal ulcers [2]. The standard recommended method to treat infected patients with severe symptoms was the combination of a proton pump inhibitor and two antibiotics, mainly clarithromycin with either amoxicillin or metronidazole [3]. An eradication rate >90% was found in a number of reports based on this combination therapy [4]. However, widespread use of antibiotics has led to a relatively high failure rate (20–40%) in the past years [5]. Antimicrobial resistance was found to be the main cause of therapy failure [6].

Taiwan has established a national health insurance system since 1995 that is controlled by the Department of Health. In February 2001, the Bureau of National Health Insurance (BNHI) of Taiwan commenced a new policy to control the use of antimicrobial agents for the treatment of acute upper respiratory infections (URIs), namely that without evidence of bacterial involvement, antibiotic costs are not reimbursed. Following this restriction, the consumption of a number of antibiotics fell, particularly the first-line antibiotics [7]. Concurrently, the National Health Research Institutes (NHRI) and national medical centres continue to survey the antimicrobial agent usage for infectious diseases as well as provide education to health professionals, which has greatly enforced the cautious usage of antimicrobial agents.

However, the resistance rates of *H. pylori* before and after the government policy were not evaluated. In this study, we retrospectively investigated subjects who received the triple therapies between 1998 and 2004 at a single medical centre to assess whether the prevalence of antimicrobial resistance and cure rates of *H. pylori* were influenced by the use of antimicrobial agents.

2. Materials and methods

2.1. Patients and bacterial culture

A total of 218 *H. pylori* isolates were collected over a period of 6 years (April 1998 to November 2004) from patients who visited Taichung Veterans General Hospital, Taichung, Taiwan, and underwent upper digestive endoscopy for the evaluation of dyspeptic

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Table 1
Number of *Helicobacter pylori* isolates resistant to each antibiotic[a].

Antibiotic	1998–2000 (n = 84)		2001–2004 (n = 134)		Total (n = 218)	P-value[b]
	M/F	All	M/F	All		
MTZ	20/15	35 (41.7%)	14/20	34 (25.4%)	69 (31.7%)	<0.05
CLR	6/3	9 (10.7%)	4/5	9 (6.7%)	18 (8.3%)	0.42
AMX	0	0	0	0	0	–

MTZ, metronidazole; CLR, clarithromycin; AMX, amoxicillin; –, no comparative data.
[a] By intention-to-treat analyses.
[b] Analysed by comparing resistance rates in 1998–2000 with 2001–2004.

symptoms. None of the patients had a previous history of *H. pylori* infection. The patients recruited in this investigation ranged in age from 21 to 78 years (mean ± standard deviation, 53.3 ± 11.5 years) and 141 patients (64.7%) were male. Bacterial strains were first isolated from patient biopsies and grown on Brucella blood agar plates (Becton Dickinson, Sparks, MD) as described previously [8].

All enrolled patients provided informed consent before beginning the experimental protocol.

2.2. Treatment of patients

Patients enrolled in the study were randomly assigned to receive one of two regimens as described previously [9]. In brief, patients in the first group were treated with lansoprazole 30 mg, clarithromycin 500 mg and amoxicillin 1 g (LAC) twice daily for 1 week. Patients in the second group were treated with lansoprazole 30 mg, clarithromycin 500 mg and metronidazole 500 mg twice daily (LMC) for 1 week. Assessment of *H. pylori* status was carried out with a ^{13}C-urea breath test and bacterial culture at diagnosis, and by ^{13}C-urea breath test at least 2 weeks after the end of therapy.

2.3. Antimicrobial susceptibility test

The *H. pylori* isolates were tested for metronidazole, clarithromycin and amoxicillin susceptibility using the Etest (AB BIODISK, Solna, Sweden) as previously described [8]. Metronidazole resistance was defined as a minimum inhibitory concentration (MIC) >8 mg/L, and amoxicillin resistance and clarithromycin resistance were defined as a MIC > 2 mg/L.

2.4. Analysis of the National Health Insurance database

The National Health Insurance database was made available for the purpose of research by contacting the NHRI [10]. A systematic sampling method was used to collect a random representative data set from the entire database. The size of the subset from each month was determined by the ratio of the amount of data in each month to that of the entire year. Systematic sampling was then performed for each month to choose randomly a representative subset. The sample database was obtained by combining the subsets from 12 months. The sample database of ambulatory care expenditures by visit was constructed first then the relevant observations in the details of the ambulatory care order were drawn out as necessary. The sample database of ambulatory care expenditures by visit was 0.2% of the entire database. All of the data regarding the consumption of various antibiotics were obtained from the NHRI. Medical diagnoses were classified by ICD-9-CM [11]; gastroenterological diseases were defined as ICD codes 531 to 535. Drug codes for amoxicillin, clarithromycin and metronidazole were obtained from the BNHI.

2.5. Statistical analysis

Comparison of treatment efficacy was performed using per-protocol analysis, which included all patients who were *H. pylori*-positive before treatment and had taken at least 80% of study medications. The relationship between *H. pylori* and cure rates was analysed by the χ^2 test with Yates's correction or by Fisher's exact test using SPSS programme version 10.1 (SPSS Inc., Chicago, IL). A P-value of <0.05 was considered statistically significant.

3. Results

3.1. Antimicrobial resistance in Helicobacter pylori

The prevalence of resistant in the 218 *H. pylori* isolates to metronidazole, clarithromycin and amoxicillin is shown in Table 1. Patients enrolled in the study were divided into two periods (1998–2000 and 2001–2004). The overall primary resistance rates were 31.7% (69/218) for metronidazole and 8.3% (18/218) for clarithromycin, whereas no isolates showed resistance to amoxicillin. It is noted that resistance to metronidazole was detected in 35/84 isolates (41.7%) in the period 1998–2000 compared with 34/134 (25.4%) during 2001–2004 (P < 0.05). There was also a lower frequency of clarithromycin resistance after the policy (6.7% vs. 10.7%), but with no statistical significance (P = 0.42).

Table 2
Distribution of primary antibiotic susceptibility of *Helicobacter pylori* and eradication rates in relation to treatment groups and primary antibiotic resistance.

	Period	MTZ^S/CLR^S	MTZ^S/CLR^R	MTZ^R/CLR^S	MTZ^R/CLR^R	Total
Antibiotic susceptibility [n (%)]						
LAC	1998–2000	27 (57.4)	3 (6.4)	16 (34.0)	1 (2.1)	47
	2001–2004	48 (68.6)	1 (1.4)	17 (24.3)	4 (5.7)	70
LMC	1998–2000	18 (48.6)	1 (2.7)	14 (37.8)	4 (10.8)	37
	2001–2004	48 (75.0)	3 (4.7)	12 (18.8)	1 (1.6)	64
Eradication rate [n (%)][a]						
LAC	1998–2000	20/21 (95.2)	0/3 (0)	12/13 (92.3)	0/1 (0)	32/38 (84.2)
	2001–2004	31/32 (96.9)	0/1 (0)	8/10 (80.0)	0/3 (0)	39/46 (84.8)
LMC	1998–2000	12/13 (92.3)	0/1 (0)	9/11 (81.8)	0/3 (0)	21/28 (75.0)
	2001–2004	32/34 (94.1)	0/3 (0)	6/8 (75.0)	0/1 (0)	38/46 (82.6)

MTZ^S, metronidazole-susceptible; MTZ^R, metronidazole-resistant; CLR^S, clarithromycin-susceptible; CLR^R, clarithromycin-resistant; LAC, lansoprazole, amoxicillin and clarithromycin; LMC, lansoprazole, metronidazole and clarithromycin.
[a] By per-protocol analysis.

Table 2 shows the distribution of primary antibiotic resistance for the two regimens in the periods of 1998–2000 and 2001–2004. There was no significant difference in either metronidazole or clarithromycin resistance between the LAC and LMC groups. To evaluate the prevalence of metronidazole resistance (with or without clarithromycin resistance), the total *H. pylori* isolates were classified into two periods, 1998–2000 and 2001–2004. As seen in Table 2, there was a lower rate of metronidazole resistance during 2001–2004 compared with 1998–2000 both in the LAC group (36.1% vs. 30.0%) and the LMC group (48.6% vs. 20.4%; $P < 0.05$). Similar results were also found for clarithromycin resistance (with or without metronidazole resistance): 8.5% (1998–2000) vs. 7.1% (2001–2004) in the LAC group and 13.5% vs. 6.3% in the LMC group. These results are in accord with those in Table 1, indicating a trend of reduced antimicrobial resistance after realising the regulation of restricted antibiotic use in Taiwan.

3.2. Helicobacter pylori eradication

Table 2 also shows the eradication rates in relation to the two regimens. By per-protocol analyses, the overall cure rate was 84.5% (71/84) and 79.7% (59/74) for the LAC and LMC groups, respectively. There was no significant difference in eradication rates between LAC and LMC. Accordingly, no significant difference was found in eradication rates in either of the two periods. Next, the cure rate between the two periods for each regimen was compared. No difference was found in the LAC group (84.2% vs. 84.8%; $P = 0.82$). However, there was a higher eradication rate in the LMC group during 2001–2004 (75.0% vs. 82.6%), but with no statistical significance ($P = 0.62$).

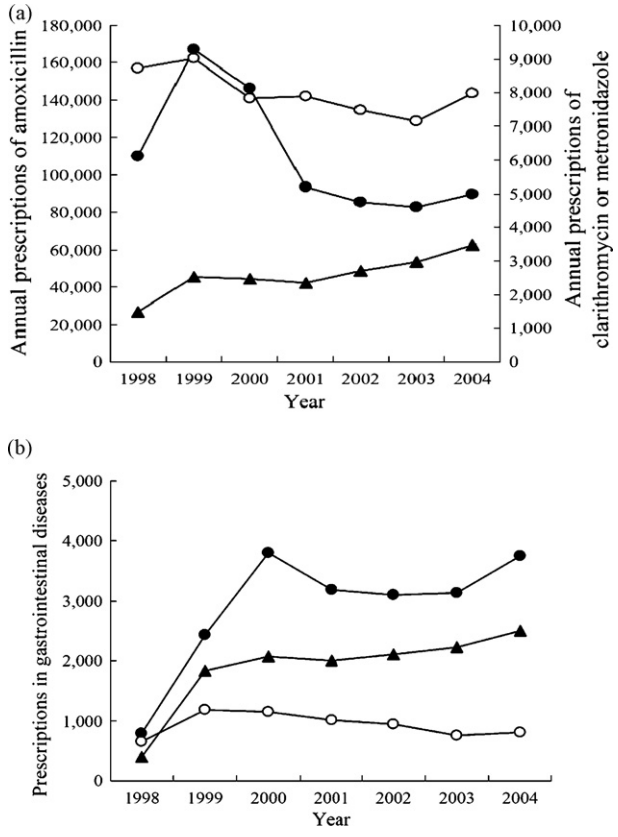

Fig. 1. (a) Total annual prescriptions for outpatients of various antibiotics and (b) annual prescriptions for treatment of gastrointestinal diseases from 1998 to 2004 in Taiwan: ●, amoxicillin; ▲, clarithromycin; and ○, metronidazole.

3.3. Decreased antimicrobial consumption reduces Helicobacter pylori resistance

In Taiwan, the total usage [defined daily doses (DDDs)/1000 population/day] of antimicrobial agents in the treatment of ambulatory patients with URI progressively reduced from 1999 to 2001. Importantly, there was a reduction of 33.1% in 2001 compared with 1999 [7]. To assess the consumptions of amoxicillin, clarithromycin and metronidazole for patients with gastrointestinal diseases, a representative data set from the NHRI database was randomly collected using the systematic sampling method. Of 19 891 246 details of ambulatory care orders, there were 200 736 prescriptions for at least one of amoxicillin, clarithromycin or metronidazole, and 4 476 485 prescriptions described as ambulatory care expenditures by visit in Taiwan from 1998 to 2004. Of 132 240 prescriptions for gastrointestinal diseases (with ICD-9-CM codes 531–535) amongst ambulatory care expenditures by visit, 3936 were for at least one of amoxicillin, clarithromycin or metronidazole. Fig. 1 shows the annual consumptions of amoxicillin, clarithromycin and metronidazole in Taiwan during 1998–2004. It is noted that the consumption of amoxicillin decreased significantly between 2000 and 2001 and gradually reduced from 2001 to 2003 in the total population (Fig. 1a) as well as in the gastrointestinal diseases population (Fig. 1b). The consumption of clarithromycin gradually increased in the gastrointestinal diseases population during 1999–2004 except for 2001, whereas that of metronidazole slowly decreased both in the total population and in the gastrointestinal diseases group during 1999–2003.

4. Discussion

In this first such single-centre Taiwanese study, *H. pylori* antimicrobial resistance and eradication rates were retrospectively investigated following two lansoprazole-based triple therapies over 6 years (1998–2004). Antimicrobial susceptibility of *H. pylori* was evaluated and it was found that the overall metronidazole resistance rate of *H. pylori* in 2001–2004 (25.4%) was much lower than in 1998–2000 (41.7%). The resistance rate for clarithromycin in *H. pylori* isolates was also slightly lower in 2001–2004 than in 1998–2000 (6.7% vs. 10.7%), but with no significant difference.

Analysis of the NHRI database shows that there is indeed a significant drop in the total usage of amoxicillin, consistent with previous results [7]. Additionally, we found that there were reduced consumptions of metronidazole in the total population as well as in the gastrointestinal disease population after the BNHI policy, even though metronidazole is not routinely prescribed to treat respiratory tract infection. These results suggest that our health professionals may acquire a general perception to reduce the use of antibiotics owing to the government rule as well as other non-legislative measures. On the other hand, the annual prescriptions of clarithromycin increased from ca. 2500 to 3500 in 1999–2004. None the less, it is noted that erythromycin, another macrolide, had a reduction of 71% from 2000 (0.447 DDDs/1000 population/day) to 2003 (0.129 DDDs/1000 population/day) in Taiwan, which led to much less erythromycin resistance in *Streptococcus pyogenes* [12]. Given that erythromycin is a first-line antibiotic, its reduced usage in URI patients might surpass the contribution from the increased consumption of clarithromycin, resulting in a lower rate of clarithromycin resistance in *H. pylori*. These data together suggest a positive link between the reduced prevalence of antimicrobial resistance and the reduced use of antibiotics in treating various diseases. This, in turn, leads to a higher eradication rate.

In Japan, the resistance rate of metronidazole has been observed to rise in a long-term survey possibly owing to its increased use from 6.6% in 1997–1998 to 12.0% in 1999–2000 [13]. Similar results were also reported in a 9-year survey in Spain prior to 2000 [14].

In contrast, a recent report from Sweden demonstrated overall reduced rates of antibiotics resistance owing to the restrictive prescribing policy to control the use of antimicrobial agents [15]. In accord with their results, we found a much lower rate of metronidazole resistance in *H. pylori* (41.7% in 1998–2000 vs. 25.4% in 2001–2004). Despite no statistical significance, our results showed that the eradication rate increased from 75.0% (1998–2000) to 82.6% (2001–2004) for the metronidazole-containing therapy.

In conclusion, this retrospective study shows that the resistance rates for clarithromycin and metronidazole of *H. pylori* in Taiwan have decreased following governmental policy to restrict the use of antimicrobial agents for infectious diseases. Such a programme may be an effective method for controlling antimicrobial resistance in *H. pylori* infection. Future studies are needed to monitor the trend in the rate of antimicrobial resistance for *H. pylori* after 2005.

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Competing interests: None declared.

Ethical approval: This study was approved by the Ethics Committee of the Taichung Veterans General Hospital, Taichung, Taiwan.

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