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Original Article

Comparison of the Antibiotic Resistance of *Helicobacter pylori* Isolated in Jinju Over a 15-year Period

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The aims of this study were to investigate the changing pattern of *Helicobacter pylori* antibiotic resistance in Jinju over a 15-year period. *H. pylori* strains were isolated from 170 adults living in Jinju from 1985-1989, 1990-1994 and 1995-1999, and from 23 adults living in Cheongju from 1995 to 1999. Susceptibility to erythromycin, clarithromycin, azithromycin, amoxicillin, tetracycline, metronidazole, furazolidone, levofloxacin, ciprofloxacin, moxifloxacin, and rifabutin was tested using the serial two-fold agar dilution method. Moxifloxacin resistance significantly increased in Jinju from 1985-1989 (0%) to 1995-1999 (14.9%) ($p < 0.0001$). Resistance to amoxicillin was increasesed trend to decreased trend from 1985 to 1999 ($p = 0.033$), whereas metronidazole resistance decreased from 37.5% to 21.3%. Resistance to furazolidone was greater from 1985-1989 (9.4%) than in 1995-1999 (2.1%). In comparing Jinju and Cheongju, minimal inhibitory concentrations (MICs) of tetracycline and levofloxacin among *H. pylori* isolated from Jinju were lower than for isolates from Cheonju ($p < 0.05$). The levofloxacin resistance rate was higher in Cheongju than in Jinju ($p = 0.02$). No macrolide resistance was observed in Cheongju. Overall, we did not observe any remarkable antimicrobial resistance increase of *H. pylori* strains isolated from Jinju over 15 years. The MIC distributions of antimicrobials and antimicrobial resistant rates were time- and region-specific among different strains. Future anti-*H. pylori* eradication regimens should be designed based on the changing patterns of antimicrobial resistance according to the resident area.

Key Words: *Helicobacter pylori*, Minimal inhibitory concentration, Antimicrobial agents, Antibiotic resistance

INTRODUCTION

Helicobacter pylori infection causes chronic gastritis, gastroduodenal ulcers, and gastric cancer. Currently, the initial management of *H. pylori* infection includes prescribing a proton pump inhibitor and a combination of two or more antibiotics such as amoxicillin, clarithromycin, or metronidazole (1~4). However, antibiotic resistance is an important problem (5, 6); *H. pylori* strains are increasingly resistant to metronidazole, clarithromycin, and amoxicillin (7, 8).

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H. pylori resistance rates can vary among groups of patients according to age, sex, disease, and place of residence. In some countries, there is an evolution of resistance that often reflects the previous or current national consumption of a given antibacterial agent (9, 10). Antibiotic resistance testing of regional *H. pylori* isolates might be helpful for predicting the outcomes of standard treatment (11, 12). The aim of this study was to investigate antibiotic resistance rates of *H. pylori* isolated in Jinju between 1985 and 1999 and to compare our findings with isolates from Cheongju collected between 1995 and 1999.

MATERIALS AND METHODS

Patients

Strains were collected from the adult patients undergoing upper gastrointestinal endoscopy to evaluate abdominal pain or gastrointestinal bleeding at Gyeongsang National University (GNU) Hospital in 1985-1989, 1990-1994, and 1995-1999 and at Chungbuk National University in 1995 -1999.

Biopsy specimens and *H. pylori* culture

Fresh antral and body biopsy specimens that were freshly obtained (used within 1 h) or kept frozen at the biobank (>1 year) were transported to the microbiology laboratory and cultured on Mueller-Hinton agar (MHA, Difco Co., Detroit, MI, USA) plates containing 10% bovine sera, vancomycin (10 μg/ml), nalidixic acid (25 μg/ml) and amphotericin B (5 μg/ml). The plates were incubated at 37℃ under microaerophilic conditions under a 10% CO_2 atmosphere at 100% humidity for 3~5 days. All frozen biopsy specimens were provided by Gyeongsang National University Hospital, a member of the National Biobank of Korea, after the Institutional Review Board reviewed the research protocols (GNUHIRB-2009-007).

The organisms were identified as *H. pylori* by colony morphology; Gram staining; and positive urease, catalase, and peroxidase tests. Once cultured, the *H. pylori* were stored and distributed by the *H. pylori* Korean Type Culture Collection at Gyeongsang National University School of Medicine.

Susceptibility tests

The minimal inhibitory concentration (MIC) values of the *H. pylori* isolates to erythromycin (Sigma Chemical Co., St. Louis, MO, USA), clarithromycin (Sigma Chemical Co.), azithromycin (Pfizer Central Research, Groton, CT, USA), amoxicillin (Sigma Chemical Co.), tetracycline (Sigma Chemical Co.), metronidazole (Sigma Chemical Co.), furazolidone (Sigma Chemical Co.), levofloxacin (Sigma Chemical Co.), ciprofloxacin (Sigma Chemical Co.), moxifloxacin (Sigma Chemical Co.), and rifabutin (Yuyu Pharma Inc., Seoul, Korea) were examined using the serial two-fold agar dilution method as described in the Clinical and Laboratory Standards Institute guidelines (13). Briefly, bacteria were subcultured on MHA supplemented with 10% defibrinated bovine sera for 48 h. The bacterial suspension was adjusted to 1×10^7 colony-forming units and was inoculated directly onto each antibiotic-containing agar dilution plate. MICs were determined after 72 h of incubation. The resistance breakpoints for clarithromycin, azithromycin, and erythromycin were all set as >1.0 μg/ml and those for amoxicillin, tetracycline, metronidazole, and furazolidone were defined as ≥0.5 μg/ml, >2.0 μg/ml, >8.0 μg/ml, and >1.0 μg/ml, respectively. The breakpoints for levoflaxacin, ciproflaxin, moxifloxacin, and rifabutin were provisionally defined as >1.0 μg/ml. Multidrug resistance was defined as resistance to >2 of the following antibiotics: macrolide, amoxicillin, tetracycline, metronidazole, furazolidone, fluoroquinolone, and rifabutin.

Statistical analysis

Data were analyzed using SPSS version 12.0 software (SPSS Inc., Chicago, IL, USA). The Wilcoxon rank-sum test and the Kruskal-Wallis tests were used for two-group and multiple-group comparisons, respectively. Chi-squared tests were used to compare sex, diagnosis, MIC distributions, and antibiotic resistance frequencies. Statistical significance was set at $p < 0.05$.

Table 1. Demographic features and underlying gastric diseases according to period and region of *H. pylori* isolation

Period (isolation area)	Age, yrs (Median age, range)	Sex		Diagnosis				
		Male (%)	Female (%)	Chronic gastritis	Nodular gastritis	Gastric ulcer	Duodenal ulcer	Gastric cancer
1985-1989 (Jinju)	30.0 (20~39)	35 (66.0)	19 (34.0)	50	0	1	3	0
1990-1994 (Jinju)	33.2 (20~61)	43 (61.4)	27 (38.6)	69	0	0	1	0
1995-1999 (Jinju)	22.7 (20~71)	33 (70.2)	14 (29.8)	36	0	9	1	0
1995-1999 (Cheongju)	42.0 (16~62)	16 (69.6)	7 (30.4)	10	1	3	8	1

Table 2. Distribution of *H. pylori* in Jinju according to the number of antibiotic resistant strains between 1985 and 1999.

Year	Number of antibiotic resistance in one *H. pylori* strain							Total (%)
	0 (%)	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)	6 (%)	
1985-1989	23 (43.4)	21 (39.6)	4 (7.5)	4 (7.5)	0 (0.0)	0 (0.0)	1 (1.9)	53 (100)
1990-1994	31 (44.3)	24 (34.3)	5 (7.1)	5 (7.1)	2 (2.9)	2 (2.9)	1 (1.4)	70 (100)
1995-1999	26 (55.3)	11 (23.4)	7 (14.9)	1 (2.1)	1 (2.1)	1 (2.1)	0 (0.0)	47 (100)
Total	80 (47.1)	56 (3)	16 (9.4)	10 (5.9)	3 (1.8)	3 (1.8)	2 (1.2)	170 (100)

RESULTS

Changing pattern of MIC and antibiotic resistance in Jinju

Strains were collected from 170 patients (111 men [65.3%] and 59 women [34.7%]) with a median age of 28.8 years (range 16.0~71.0). Patients were diagnosed with gastritis (n = 155), gastric ulcer (n = 10), or duodenal ulcer (n = 5) (Table 1). There were no differences in distribution of sex or age or underlying disease among the three collection periods.

Resistance to moxifloxacin was significantly increased from 1985-1989 (0%) to 1995-1999 (14.9%) ($p < 0.0001$). The amoxicillin resistant rate was 7.5% in 1985-1989, 24.3% in 1990-1994, and 12.8% in 1995-1999. Resistance to amoxicillin in the 1990-1994 was significantly different from the other time points ($p = 0.033$) (Fig. 1).

There were no significant changes in other antibiotic resistances among *H. pylori* isolated from 1985-1999 in Jinju. Resistances to clarithromycin, azithromycin, and erythromycin were 1.9%, 7.5%, and 3.8% in 1985-1989; 5.7%, 7.1%, and 12.8% in 1990-1994; and 3.8%, 2.9%, and 10.6% in 1995-1999. Resistance to tetracycline showed a similar pattern to amoxicillin (9.4%, 17.1%, and 12.8% in 1985-1989, 1990-1994, and 1995-1999, respectively). Resistance to metronidazole decreased from 37.7% to 21.3% ($p = 0.251$) (Fig. 1). Resistance to furazolidone declined from 9.4% in 1985-1989 to 2.1% in 1995-1999. Rifabutin resistance remained low throughout (<5.0% in both 1985-1989 and 1995-1999).

The percentage of isolates that were resistant to >1 antibiotic was 56.6% in 1985-1989, 55.7% in 1990-1994, and 44.7% in 1995-1999 ($p = 0.409$). Multidrug resistance rate was 17.0% in 1985-1989, 21.4% in 1990-1994, and 21.3% in 1995-1999 ($p = 0.803$) (Table 2).

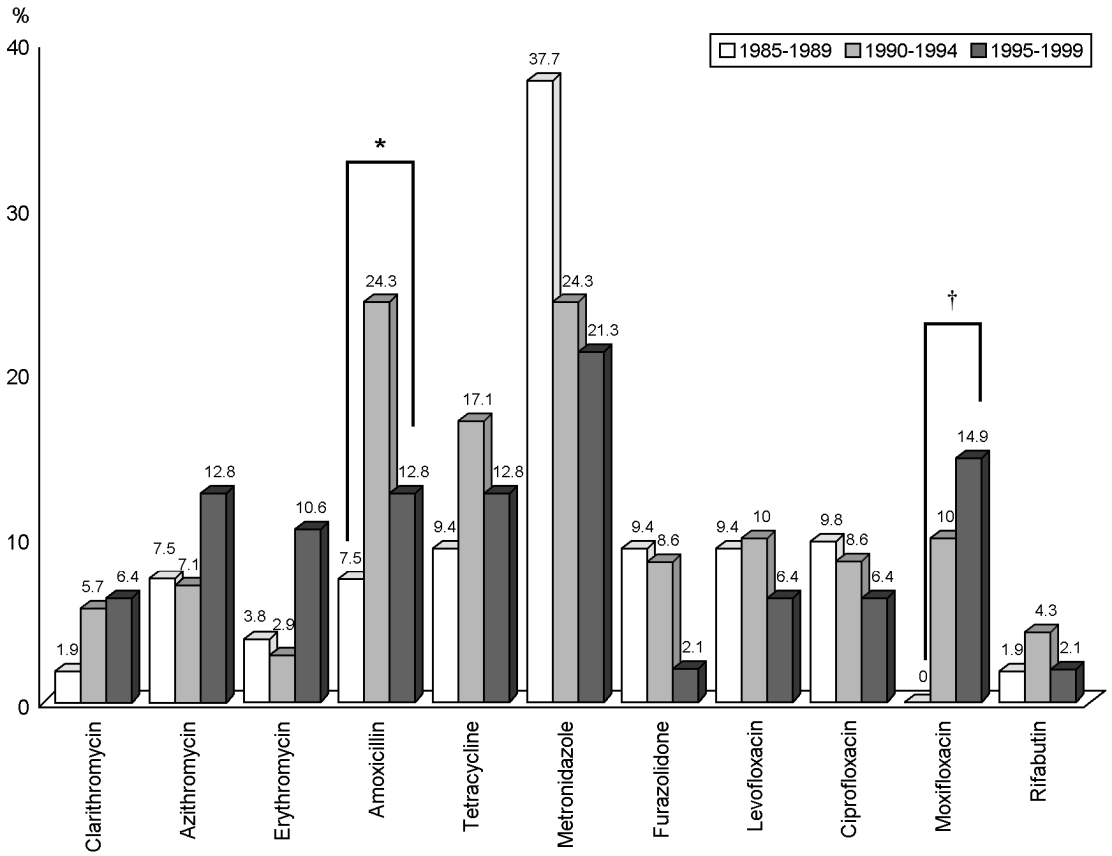

Figure 1. Antibiotic resistance rates of *H. pylori* isolated in Jinju from 1985 to 1999. Moxifloxacin resistance was significantly increased from 1985-1989 (0%) to 1995-1999 (14.9%), and resistance to amoxicillin was significantly changed from 1985 to 1999. $^{*}p < 0.0001$, $^{\dagger}p = 0.033$

Comparing antibiotic resistance in Jinju and in Cheongju

Strains were collected from 23 patients (16 men [69.6%] and 7 women [30.4%]) in Cheongju during 1995-1999, with a median age of 42.0 years (range 16.0~62.0). Patients were diagnosed with gastritis (n = 10), nodular gastritis (n = 1), gastric ulcer (n = 3), duodenal ulcer (n = 8), or gastric cancer (n = 1) (Table 1). Antibiotic resistance was compared with 47 *H. pylori* strains collected in Jinju between 1995 and 1999. MIC distributions of tetracycline (Fig. 2) and levofloxacin (Fig. 3) in *H. pylori* isolated from Jinju were lower than in those from Cheongju ($p < 0.05$).

The levofloxacin resistance rate was higher in Cheongju (26.1%) than in Jinju (6.4%, $p = 0.023$) (Fig. 4). No macrolide resistance was observed in *H. pylori* strains isolated from Cheongju. There were no statistical differences of macrolide resistance between Jinju and Cheongju. Resistances to amoxicillin, tetracycline, metronidazole, furazolidone, ciprofloxacin, and moxifloxacine were higher in Cheongju than in Jinju, but these differences were not statistically significant (Fig. 4). Rifabutin resistance was not observed in Cheongju.

Out of 69 strains, 36 (52.2%) were resistant to one or more antibiotics, and the antibiotic resistance rate was statistically different between Jinju (43.5%) and Cheongju (69.6%, $p = 0.041$). The prevalence of strains with resistance to ≥2 antibiotics was 23.9% in Jinju and 34.8% in Cheongju (Table 3), and these values were not significantly different ($p = 0.341$).

In Jinju, triple resistance to macrolide, amoxicillin and metronidazole was found in two strains among 11 strains

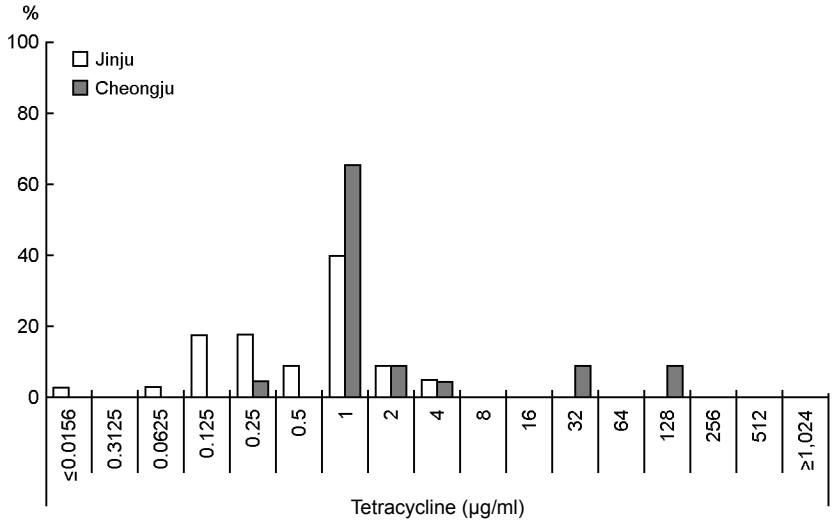

Figure 2. MIC distributions of tetracycline for *H. pylori* isolated in Jinju and Cheonju. MIC distributions of tetracycline among *H. pylori* isolated from Jinju were lower than for those from Cheongju.

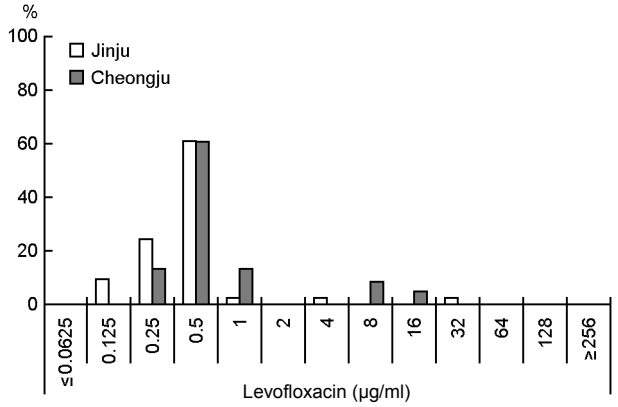

Figure 3. MIC distributions of levofloxacin for *H. pylori* isolated in Jinju and Cheonju. MIC distributions of levofloxacin among *H. pylori* isolated from Jinju were lower than for those from Cheongju.

with resistance to ≥2 antibiotics. Three of 5 strains resistant to marolide were also resistant to amoxicillin or metronidazole. In Cheongju, five of the 6 strains resistant to amoxicillin were also resistant to tetracycline. Four to the 6 strains resistant to amoxicillin were also resistant to quinolone. Dual resistance to amoxicillin and metronidazole was found in two strains among 9 strains with resistance to ≥2 antibiotics.

DISCUSSION

The present study showed no remarkable increment of antimicrobial resistant rates of *H. pylori* strains isolated over 15-year period in Jinju. The distribution of MICs of amoxicillin, metronidazole, and tetracycline also showed no significant changes during the same periods. Resistance to moxifloxacin increased with time and the resistance to amoxicillin changed (increasing trend from 1985-1989 to 1990-1994 and then decreasing trend from 1990-1994 to 1995-1999). These results might be related with that moxifloxacin was introduced since 1990s and replaced the amoxicillin. In Seoul from 1987 to 2003, the MICs of amoxicillin, clarithromycin, metronidazole, tetracycline, azithromycin, and ciprofloxacin for *H. pylori* strains increased and rates to clarithromycin increased from 2.8% to 13.8% (14). Seoul is capital huge and crowded city having 10 million in Korea. Jinju is small city having 350, 000, located in the west Gyeongnam Province. Population movement is little in Jinju. Little changes of MICs and resistance of antibiotics were related to less movement of citizen and less change of doctors living in Jinu.

Comparing the antibiotic MICs and resistances of Cheongju and Jinju, those were different during same period. Different resistance rates to metronidazole, levofloxacin, and moxifloxacin reported between 2 institutions located in Seoul and Gyeonggi province and institutional difference of antibiotic resistance of *H. pylori* resulted in difference of eradication rate of *H. pylori* according to institution (15).

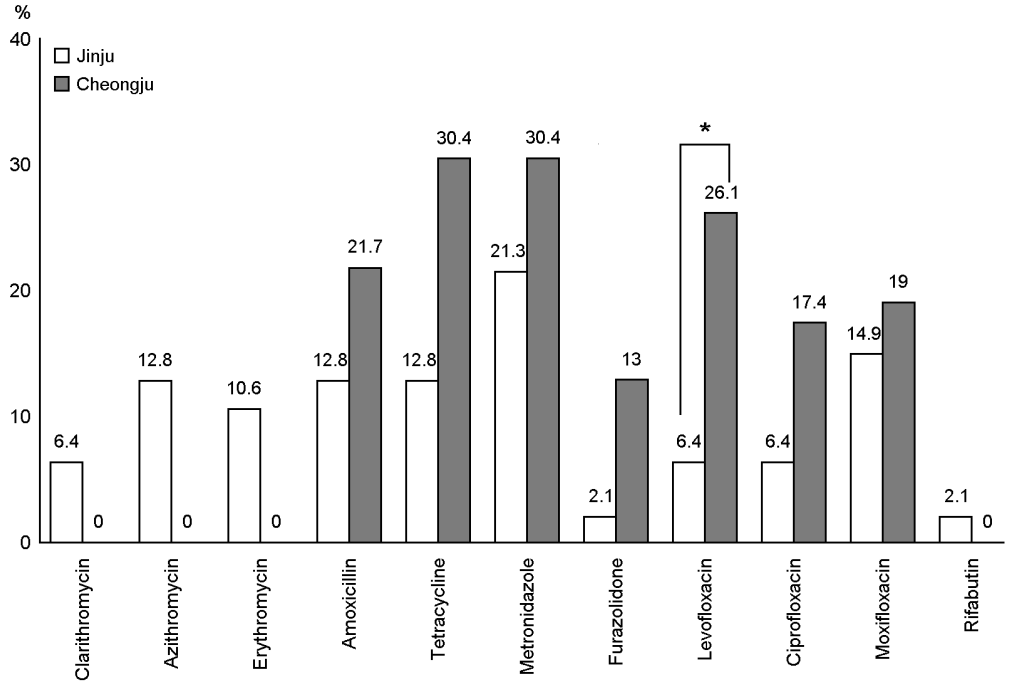

Figure 4. *H. pylori* antibiotic resistance in Jinju and Cheongju. Levofloxacine resistance was significantly different. $^{*}p = 0.023$

Table 3. *H. pylori* distribution according to the number of antibiotic resistant strains in Jinju and Cheongju.

Area	Number of antibiotic resistance in one *H. pylori* strain						Total (%)
	0 (%)	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)	
Jinju	26 (56.5)	9 (19.6)	8 (17.4)	1 (2.2)	1 (2.2)	1 (2.2)	46 (100)
Cheongju	7 (30.4)	8 (34.8)	5 (21.7)	0 (0.0)	2 (8.7)	1 (4.3)	23 (100)
Total	33 (47.8)	17 (24.6)	13 (18.8)	1 (1.4)	3 (4.4)	2 (3.0)	1 (100)

Cheongju is bigger than Jinju and capital of Chungbuk Province, near to Seoul and Gyeonggi Province. The resistances of amoxicillin, tetracycline, metronidazole, and levofloxacin in Cheongju were more similar to Seoul than in Jinju. The difference of antibiotic resistance between Cheongju and Jinju might be resulted from different prescription of antibiotics in resident doctors.

International Guidelines have recommended a 7-day triple therapy consisting of clarithromycin, and either metronidazole or amoxicillin, with a proton pump inhibitor or ranitidine bismuth citrate as a first-line treatment for curing *H. pylori* infection (16~18). Clarithromycin is the drug of choice of chemotherapy for *H. pylori* infection. Macrolide resistance is important for management of *H. pylori* infection. Rates of primary resistance to clarithromycin increased from 2.8% in 1994 to 13.8% in 2003 (19). Resistance to clarithromycin increased in *H. pylori* isolated from patients in Jinju but increasing rate of clarithromycin resistance was lower than other reports in Korea (14, 20, 21). The range of macrolide resistance was 6.4~12.8% in Jinju, but no resistance to macrolides was observed in Cheoungju. Amoxicillin resistance was 21.7% in Cheongju and it is higher than in Jinju. This result suggested that clarithromycin-amoxicillin regimen is more effective than metronidazole-amoxicillin regimen in Cheongju. Previous reports in Korea, resistance to metronidazole was high, 50%

(14). In Jinju, resistance rate to metronidazole decreased but there was no support with statistics. This results suggested that metronidazole and amoxicillin based chemotherapy is still effective regimen in Jinju, but the standard 7-day clarithromycin containing regimen is not valid as the empirical first-line eradication therapy in Jinju. Levofloxacin resistance is higher in Cheongju compared with that in Jinju. The high rate of resistance to levofloxacin may reflect that this antibiotic has already been extensively used in Cheongju.

In recent Korean study, *H. pylori* eradication rates of rifabutin- or levofloxacin-based triple therapy could not achieve enough eradication rate (22). The antibiotic resistances in Cheongju suggested that levofloxacin-based triple therapy would be low eradication rate as like the result. However, resistance to rifabutin was very low in both cities and no increment for 15-year period in Jinju. Further study is needed to assess the efficacy of rifabutin-based triple therapy for a rescue therapy in *H. pylori* infection.

There are some limitations to the present study including the small and different numbers of *H. pylori* isolates from Jinju and Cheongju, the different age distributions from Jinju and Cheonju, and no comparison with recently isolated *H. pylori*. The lately isolated *H. pylori* were obtained in 1999 and it is too old to provide valuable information for the current standard treatment. However, this result might be helpful to investigate the changing pattern of antimicrobial resistance in *H. pylori* isolated in Jinju and to compare the antimicrobial resistance in recently isolated *H. pylori* isolated in Jinju.

In conclusion, the MIC distributions of antimicrobial agents against *H. pylori* and antimicrobial resistant rates were different among strains depending on the regions of isolation. Pretreatment microbial susceptibility testing is highly recommended for success in eradication of *H. pylori* infection. In addition, changing pattern of antibiotic sensitivity should be identified according to region.

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