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Impact of Clarithromycin Resistance on Eradication of *Helicobacter pylori* in Infected Adults

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The outcome of *Helicobacter pylori* infection was analyzed in 114 dyspeptic patients treated with triple-drug therapy including clarithromycin. Clarithromycin resistance (in 20.2% of our isolates) was mainly caused by an A2142G mutation in the 23S rRNA gene of *H. pylori*. *H. pylori* eradication was obtained in all patients with clarithromycin-susceptible isolates but not in any patients with clarithromycin-resistant isolates ($P = 0.0001$). Therefore, it would be useful to conduct *H. pylori* antimicrobial susceptibility testing of the first gastric biopsy culture before choosing the first three drugs for therapy of infected patients.

Helicobacter pylori infects about 50% of the world's population and is thus a major source of gastritis, gastric ulcer, and duodenal ulcer and an important risk factor for gastric cancer (11, 12, 21). In the Republic of Korea, it is estimated that about 60 to 70% of adults are infected with *H. pylori* (8). A recent consensus statement on *H. pylori* infection (3) indicates that gastrointestinal endoscopy with a biopsy is the preferred method of investigation for patients with digestive symptoms suggestive of organic disease. It is widely accepted that all patients with gastric or duodenal ulcer and *H. pylori* infection should be treated with antimicrobial agents, since eradication of the bacteria cures peptic ulcer disease and efficiently prevents relapses (3). For adults, the recommended triple-drug therapies consist of a proton pump inhibitor (PPI) (omeprazole) together with amoxicillin and clarithromycin or metronidazole (6). Metronidazole resistance is high (50% in Europe), and *H. pylori* eradication rates are lower for adults infected with a metronidazole-resistant isolate than for those infected with a susceptible one (1). Furthermore, the presence of CagA (cytotoxin-associated gene)-positive isolates leads to difficulties in bacterial eradication (14). The consensus statement (3) proposes a follow-up strategy for those who remain infected after a first-cure treatment, i.e., a second endoscopy with culture and antimicrobial susceptibility testing and adapting the treatment. In contrast, no proposal is made recommending primary culture and antimicrobial susceptibility before first-line treatment for infected adults. Clarithromycin-resistant *H. pylori* isolates are becoming increasingly prevalent, although it is not clear to what extent these resistant organisms will spread and cause treatment failure of patients with peptic ulcers.

First, we analyzed the prevalence and mechanism of clarithromycin resistance in *H. pylori* strains isolated from infected adults with peptic ulcers before and after eradication therapy. Second, we examined the effect of clarithromycin resistance on the eradication of *H. pylori* in the patients and evaluated the usefulness of testing *H. pylori* antimicrobial susceptibility in the first gastric biopsy culture before choosing the appropriate treatment for patients. The CagA expression status of all patients was determined in order to avoid bias in the interpretation of results.

This study was a prospective study of a single center; a total of 114 patients with symptoms of dyspepsia undergoing upper gastrointestinal endoscopy at the Research Center of Gastroenterology of the Dankook University Hospital in Cheonan, Republic of Korea, between January 2000 and June 2002 were studied. Informed consent was obtained from all patients. Characteristics of the patients are shown in Table 1. The inclusion criteria were the presence of gastric or duodenal *H. pylori* infection as documented by histology, the rapid urease test (RUT), detection of the 26-kDa cell surface antigen (*SSA*) gene of *H. pylori*, and a positive culture for *H. pylori* isolates. All patients underwent endoscopy, and three biopsies taken from the antrum were used for a RUT (CLO test; Delta West Pty, Ltd., Bentley, Western Australia, Australia), histology, and culture. For histological examination, biopsy specimens were immediately fixed in buffered neutral formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin stain and modified Giemsa stain (Difco Laboratories, Detroit, Mich.) for the detection of *H. pylori*. The exclusion criteria included the following: age <18 or >90 years; treatment with a PPI (omeprazole, lansoprazole, or pantoprazole), H_2 blocker (cimetidine, ranitidine, nizatidine, famotidine, or roxatidine), and/or antibiotics, including clarithromycin, metronidazole, or amoxicillin during the 8 weeks preceding the study; gastrointestinal malignancy; severe concomitant diseases; history of allergy; previous gastric surgery; pregnancy or lactation; alcohol abuse; drug addiction; chronic use of corticosteroids or nonsteroidal anti-inflammatory drugs.

A total of 114 isolates of *H. pylori* were cultured from biopsy specimens taken during endoscopy. Antral biopsy specimens were cultured on selective medium (Helicobacter selective agar plus 7% defibrinated horse blood; Becton Dickinson,

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TABLE 1. Characteristics of the 114 patients referred for endoscopy

Characteristic[a]	No. of patients	% of patients
Age (yr)		
≤30	27	23.7
31–40	29	25.4
41–50	30	26.3
>50	28	24.6
Sex		
Male	63	55.3
Female	51	44.7
Indications for endoscopy		
Duodenal ulcer	64	56.1
Gastric ulcer	50	43.9
RUT	114 (positive)	100
PCR		
SSA	114 (positive)	100
cagA	59 (positive)[b]	51.8
	55 (negative)	48.2

[a] RUT detects *H. pylori.* PCR was performed for *SSA*, the 26-kDa cell surface antigen gene of *H. pylori*, and *cagA*, the cytotoxin-associated gene encoding the virulence factor of *H. pylori.*

[b] According to Western blot analysis, CagA protein was expressed in all *cagA* gene-positive *H. pylori* isolates.

Cockeysville, Md.). The plates were incubated for 5 to 7 days at 37°C in microaerobic conditions (Campy Pak Plus; BBL, Becton Dickinson) and 100% relative humidity. Isolated strains were frozen at −70°C in brucella broth (Difco Laboratories). The MICs of amoxicillin (Sigma, St. Louis, Mo.) and clarithromycin (Abbott Laboratories, Queensborough, Kent, United Kingdom) were determined by the Etest (AB Biodisk, Uppsala, Sweden). Suspensions adjusted to a turbidity approximating the turbidity of a McFarland standard of 2 were used. Plates were incubated for 72 h at 37°C under microaerobic conditions. Isolates were considered resistant to amoxicillin and clarithromycin if the amoxicillin and clarithromycin MICs were above 0.5 and 1.0 μg/ml, respectively (10). *H. pylori* ATCC 43505 was used as a control. The molecular method used for diagnosis of *H. pylori* infection was PCR amplification of a fragment of the *SSA* gene from *H. pylori* isolates by the method of Mikula et al. (9) with designed primer pairs (Table 2). The detection of *cagA* gene and CagA protein from *H. pylori* isolates was performed by the PCR amplification method (23) using designed primer pairs (Table 2) and the Helicoblot 2.0 Western blot kit (Genelabs, Singapore, Republic of Singapore).

It has been shown that resistance to clarithromycin often results from G2115A, G2141A, A2142G, A2142C, A2143G, A2144T, T2182C, and/or T2717C mutations in the 23S rRNA gene of *H. pylori* (4, 7, 16, 20). Point mutations in the 23S rRNA gene were analyzed by PCR-restriction fragment length polymorphism (RFLP). Genomic DNAs of *H. pylori* isolates were prepared by using the Wizard Genomic DNA purification kit (Promega, Madison, Wis.), and these DNAs were used as template DNA in PCR with the designed primers (Table 2). Amplified DNA fragments of 1,204 bp were digested with MboII and BsaI restriction enzymes, allowing detection of A2142G and A2143G mutations, respectively. In order to detect point mutations other than A2142G and A2143G and to confirm A2142G and A2143G mutations, amplicons were analyzed by direct sequencing of both strands with an automated DNA sequencer (ABI PRISM3100; Applied Biosystems, Weiterstadt, Germany).

All adults included in the study had tested positive for *H. pylori* by histology, culture, RUT, and PCR detection of the *SSA* gene. They received a PPI, omeprazole (20 mg twice a day [BID]), together with amoxicillin (1 g BID) and clarithromycin (500 mg BID) for 10 days. Six weeks after the end of treatment, clinical symptoms were evaluated, and another endoscopy was performed, with biopsy specimens taken for histological, bacteriological, biochemical, and molecular tests. *H. pylori* eradication was defined as negative results by all four tests.

Statistical analysis was performed using SPSS software, version 11.5 for Windows (SPSS, Inc., Chicago, Ill.). Differences in the bacterial eradication rate of treatment groups were assessed by the chi-square test of homogeneity for categorical variables (χ^2 test), with P values of <0.05 considered statistically significant.

A total of 114 patients fulfilled the inclusion criteria and were enrolled in the study. Active gastric and duodenal ulcers were diagnosed in 64 (56.1%) and 50 patients (43.9%), respectively (Table 1). RUT and PCR amplification of the *SSA* gene revealed that all patients were infected with *H. pylori* (Table 1). PCR amplification of the *cagA* gene showed that 59 of the 114 (51.8%) patients were *cagA* positive and *cagA*-positive isolates expressed CagA protein (128 kDa) (Table 1). Bacteriological eradication occurred in 42 of the 59 (71.2%) CagA-positive

TABLE 2. Nucleotide sequences of the oligonucleotides used for PCR amplification and DNA sequencing

Primer (orientation)[a]	Sequence (5′ → 3′)	Position	Amplicon size (bp)	PCR primer pair[e]
SSA-F[b] (F)	TGGCGTGTCTATTGACAGCGAG	220	303	SSA-F/SSA-R
SSA-R[b] (R)	CCTGCTGGGCATACTTCACCAT	522		
CagA-F[c] (F)	AGTAAGGAGAAACAATG	14824	1320	CagA-F/CagA-R
CagA-R[c] (R)	AATAAGCCTTAGAGTCTTTTTGGAAAT	16143		
Cla-F[d] (F)	AGTATTCTAAGGCGCGTGAAAG	1743	1204	Cla-F/Cla-R
Cla-R[d] (R)	GACCTGCATGAATGGCGTAAC	2946		

[a] The orientation of each primer is shown as follows: F, forward; R, reverse.

[b] Designed according to the sequence of the 26-kDa cell surface antigen gene (*SSA;* GenBank accession no. M55507) from *H. pylori.*

[c] Designed according to the sequence of the cytotoxin-associated gene A (*cagA;* GenBank accession no. AF282853) from *H. pylori.*

[d] Designed according to the sequences of the *H. pylori* 23S rRNA gene (GenBank accession no. U27270) associated with clarithromycin (Cla) resistance.

[e] Primer pair for PCR amplification.

TABLE 3. Relationship between A-to-G mutations of clarithromycin-resistant *H. pylori* isolates and the failed eradication after clarithromycin-based therapy against 114 patients with gastric or duodenal ulcers

Isolate[a] (no.)	No. of patients[b] (%)	No. of isolates with eradication failure (%)	MIC (μg/ml)[b]	
			Amoxicillin	Clarithromycin
Wild type (91)	91 (79.83)	0 (0.00)	0.016–0.063 (S)	0.031–0.125 (S)
A2142G (20)	20 (17.54)	20[c] (17.54)	0.031–0.125 (S)	32–>256 (R)
A2143G (3)	3 (2.63)	3[c] (2.63)	0.016–0.125 (S)	4–128 (R)

[a] Sequence and PCR-RFLP analyses of the 23S rRNA gene from each *H. pylori* isolated from each patient revealed mutations (A→G) at residues 2142 and 2143.
[b] Results presented for each isolate before treatment are the same as those after treatment. Abbreviations: S, susceptible; R, resistant.
[c] Significantly different ($P = 0.0001$) from the value for wild-type isolates.

patients and 41 of the 55 (74.5%) CagA-negative patients ($P = 0.1$).

Since all clarithromycin-susceptible *H. pylori* isolates were eradicated, only clarithromycin-resistant isolates were available for MIC tests after treatment. Clarithromycin-resistant *H. pylori* isolates (MIC of >1.0 μg/ml) before and after treatment were detected in 23 of 114 patients (20.2%). Clarithromycin-resistant *H. pylori* isolates after treatment were from the same patients that had clarithromycin-resistant *H. pylori* isolates before treatment, and all clarithromycin-resistant isolates were sensitive to amoxicillin (MIC of <0.5 μg/ml) (Table 3). In other words, the incidence of primary resistance was 20.2%, and the incidence of secondary resistance (resistance in patients with *H. pylori* infection in whom a previous triple-drug therapy failed to eradicate the infection) was 100%. Clinical signs and symptoms were consistent with posttreatment biopsy results, and patients with *H. pylori*-positive posttherapy cultures still had symptoms. No correlation was found between clarithromycin resistance and patient age, sex, or the presence of gastric or duodenal ulcer. Analysis of 23S rRNA gene mutations by PCR-RFLP was performed for all *H. pylori* isolates. A2142G mutations with complete cleavage by MboII were detected in 20 of the 23 (87.0%) clarithromycin-resistant isolates. A2143G mutations with complete cleavage by BsaI were detected in 3 of the 23 (13.0%) clarithromycin-resistant isolates (Table 3). Point mutations other than A2142G and A2143G were not detected. These results were confirmed by sequence analyses of all amplicons.

More precisely, *H. pylori* eradication occurred in 79.8% of the patients in all adults infected with clarithromycin-sensitive isolates versus in none of the patients with clarithromycin-resistant isolates (Table 3). The bacterial eradication failure rate in A2142G and A2143G mutant groups was the same, and bacterial eradication failure correlated completely with the two mutations (100% of each mutant group versus 0% of wild-type group; $P = 0.0001$).

This is the first study investigating the usefulness of *H. pylori* antimicrobial susceptibility testing in the first gastric biopsy culture and the lack of efficacy of clarithromycin in cases of resistance to this drug in the Republic of Korea. The incidence of clarithromycin resistance is considerably higher than those reported for northeastern Italy (13), The Netherlands (19), and Germany (22) but was similar to the incidence previously reported for southern Italy (18). The incidence of clarithromycin resistance before clarithromycin-based triple-drug therapy was identical to that after triple-drug therapy, suggesting that there is no acquired resistance due to the triple-drug therapy. These data agree with the results of a similar study in Italy (6).

H. pylori resistance to clarithromycin correlated completely with A2142G and A2143G transition mutations in the 23S rRNA gene, resulting in a decrease in the affinity of clarithromycin binding to ribosomes. The high incidence of clarithromycin resistance in adults may reflect frequent prescriptions of macrolides for treatment of respiratory tract infections other than *H. pylori*. Some investigators have recently reported the predominance of an A2143G mutation in primary resistance isolates (20). This predominance was not shown in our study, and the A2142G mutation was predominant. Since the *H. pylori* genome is known to contain a high degree of genetic variability (15), Korean *H. pylori* isolates may be different from Western isolates of *H. pylori*.

Clarithromycin resistance was 100% predictive of treatment failure. In contrast to our data, another study (2) reported that clarithromycin resistance reduced effectiveness by an average of 55%. The differences between these rates of eradication can be explained by the different breakpoints used and different mutations involved in the mechanism of resistance. High levels of resistance cannot be dealt with by increasing the dose or duration of therapy, as previously reported by Graham and Qureshi (5). Treatment failure should prompt endoscopy, culture, and susceptibility testing. Retreatment should exclude antibiotics with acquired resistance. Many studies have highlighted the difficulties of retreatment, and it can be stated that the best available first-line treatment regimen is still the best rescue treatment (6). The *H. pylori* eradication rate was significantly improved when antibiotic therapy was performed on the basis of the results of antimicrobial susceptibility testing (18 and 17% improvement in our preliminary results and another report [17], respectively).

We conclude that there is a significant difference in bacterial eradication rate in patients infected with clarithromycin-resistant *H. pylori* isolates and clarithromycin-sensitive ones. These results do not appear to be biased by any differences in pathogenicity because of the similar CagA expression statuses of the isolates in both groups. We also suggest that *H. pylori* antimicrobial susceptibility testing of the first gastric biopsy culture is useful before choosing the first triple-drug therapy in infected patients and that clarithromycin should not be used to treat cases of primary resistance.

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REFERENCES

1. **Debets-Ossenknopp, Y. J., A. J. Herscheid, R. G. Pot, E. J. Kuipers, J. G. Kusters, and C. M. Vandenbroucke-Grauls.** 1999. Prevalence of *Helicobacter pylori* resistance to metronidazole, clarithromycin, amoxicillin, tetracycline, and trovafloxacin in The Netherlands. J. Antimicrob. Chemother. **43:**511–515.
2. **Dore, M. P., G. Leandro, G. Realdi, A. R. Sepulveda, and D. Y. Graham.** 2000. Effect of pretreatment antibiotic resistance to metronidazole and clarithromycin on outcome of *Helicobacter pylori* therapy. Dig. Dis. Sci. **45:**68–76.
3. **Drumm, B. S., S. Koletzko, and G. Oderda, on behalf of the European Pediatric Task Force on** *Helicobacter pylori*. 2001. *Helicobacter pylori* infection in children: a consensus statement. J. Pediatr. Gastroenterol. Nutr. **30:**207–213.
4. **Fontana, C., M. Favaro, S. Minelli, A. A. Criscuolo, A. Pietroiusti, A. Galante, and C. Favalli.** 2002. New site of modification of 23S rRNA associated with clarithromycin resistance of *Helicobacter pylori* clinical isolates. Antimicrob. Agents Chemother. **46:**3765–3769.
5. **Graham, D. Y., and W. A. Qureshi.** 2000. Antibiotic-resistant *H. pylori* infection and its treatment. Curr. Pharm. Des. **15:**1537–1544.
6. **Kalach, N., P. H. Benhamou, F. Campeotto, M. Bergeret, C. Doupont, and J. Raymond.** 2001. Clarithromycin resistance and eradication of *Helicobacter pylori* in children. Antimicrob. Agents Chemother. **45:**2134–2135.
7. **Khan, R., S. Nahar, J. Sultana, M. M. Ahmad, and M. Rahman.** 2004. T2182C mutation in 23S rRNA is associated with clarithromycin resistance in *Helicobacter pylori* isolates obtained in Bangladesh. Antimicrob. Agents Chemother. **48:**3567–3569.
8. **Kim, J. H., H. Y. Kim, N. Y. Kim, S. W. Kim, J. G. Kim, J. J. Kim, J. K. Seo, J. G. Sim, I. H. Roe, H. S. Ahn, B. C. Yoon, S. W. Lee, Y. C. Lee, I. S. Chung, H. Y. Jung, W. S. Hong, and K. W. Choi.** 2000. Seroprevalence of *Helicobacter pylori* infection in asymptomatic people in Korea. Kor. J. Med. **59:**388–397.
9. **Mikula, M., A. Dzwonek, K. Jagusztyn-Krynicka, and J. Ostrowski.** 2003. Quantitative detection for low levels of *Helicobacter pylori* infection in experimentally infected mice by real-time PCR. J. Microbiol. Methods **55:**351–359.
10. **National Committee for Clinical Laboratory Standards.** 1999. Performance standards for antimicrobial susceptibility testing. Ninth informational supplement M100-S9. National Committee for Clinical Laboratory Standards, Wayne, Pa.
11. **NIH Consensus Development Panel on** *Helicobacter pylori* in Peptic Ulcer Disease. 1994. *Helicobacter pylori* in peptic ulcer disease. JAMA **272:**65–69.
12. **Parsonnet, J., G. D. Friedman, D. P. Vandersteen, Y. Chang, J. H. Vogelman, N. Orentreich, and R. K. Sibley.** 1991. *Helicobacter pylori* infection and the risk of gastric carcinoma. N. Engl. J. Med. **325:**1127–1131.
13. **Pilotto, A., M. Rassu, G. Leandro, M. Franceschi, and F. Di Mario.** 2000. Prevalence of *Helicobacter pylori* resistance to antibiotics in Northeast Italy: a multicentre study. Dig. Liver Dis. **32:**763–768.
14. **Raymond, J., N. Kalach, M. Bergeret, P. H. Benhamou, J. P. Barbet, D. Gendrel, and C. Dupont.** 1998. Effect of metronidazole resistance on bacterial eradication of *Helicobacter pylori* in infected children. Antimicrob. Agents Chemother. **42:**1334–1335.
15. **Taylor, D. E., M. Eaton, N. Chang, and S. M. Salama.** 1992. Construction of *Helicobacter pylori* genome map and demonstration of diversity at the genome level. J. Bacteriol. **174:**6800–6806.
16. **Toracchio, S., G. M. Aceto, R. Mariani-Costantini, P. Battista, and L. Marzio.** 2004. Identification of a novel mutation affecting domain V of the 23S rRNA gene in *Helicobacter pylori*. Helicobacter **9:**396–399.
17. **Toracchio, S., L. Cellini, D. I. Campli, G. Cappello, M. G. Malatesta, A. Ferri, A. F. Ciccaglione, L. Grossi, and L. Marzio.** 2000. Role of antimicrobial susceptibility testing on efficacy of triple therapy in *Helicobacter pylori* eradication. Aliment. Pharmacol. Ther. **14:**1639–1643.
18. **Toracchio, S., and L. Marzio.** 2003. Primary and secondary antibiotic resistance of *Helicobacter pylori* strains in central Italy during the years 1998–2002. Dig. Liver Dis. **35:**541–545.
19. **Tucci, A., O. Varoli, R. Corinaldesi, V. Stanghellini, S. Gasperoni, G. F. Paparo, M. Ricci-Maccarini, M. La Placa, and L. Barbara.** 1993. Evaluation of *Helicobacter pylori* sensitivity to amoxicillin and metronidazole in dyspeptic patients. Ital. J. Gastroenterol. **25:**65–67.
20. **van Doorn, L.-J., Y. Glupczinski, J. G. Kusters, F. Mégraud, P. Midolo, N. Maggi-Solcà, D. M. M. Queiroz, N. Nouhan, E. Stet, and W. G. V. Quint.** 2001. Accurate prediction of macrolide resistance in *Helicobacter pylori* by a PCR line probe assay for detection of mutations in the 23S rRNA gene: multicenter validation study. Antimicrob. Agents Chemother. **45:**1500–1504.
21. **Warren, J. R., and B. J. Marshall.** 1983. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet **i:**1273–1275.
22. **Wolle, K., M. Nilius, A. Leodolter, W. A. Muller, P. Malfertheiner, and W. Koning.** 1998. Prevalence of *Helicobacter pylori* resistance to several antimicrobial agents in a region of Germany. Eur. J. Clin. Microbiol. Infect. Dis. **17:**519–521.
23. **Xiang, Z., S. Censini, P. F. Bsyeli, Y. Chang, J. H. Vogelman, N. Orentrich, and R. K. Sibley.** 1995. Analysis of expression of *cagA* and *vacA* virulence factors in 43 strains of *Helicobacter pylori* reveals that clinical isolates can be divided into two major types and that *cagA* is not necessary for expression of the vacuolating cytotoxin. Infect. Immun. **63:**94–98.