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Article

Multiple Antibiotic Resistance of *Helicobacter pylori* and the Difficulty of *Helicobacter pylori* Eradication

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Abstract: Eradication of *Helicobacter pylori* (*H. pylori*) is extremely important to reduce the risk of developing gastric ulcer and gastric cancer. However, in recent years, a decrease in the success rate of eradication has been reported due to an increase in antibiotic-resistant bacteria. In particular, there is concern in Japan about the impact of resistance to clarithromycin (CAM), metronidazole (MNZ), and amoxicillin (AMPC), which are used for primary and secondary eradication, on the difficulty of eradication. In this study, we investigated the susceptibility to AMPC, CAM, and MNZ in patients presenting to a university hospital for *H. pylori* eradication and evaluated the impact of each resistance pattern on the eradication success rate using logistic regression analysis. The results showed that the eradication success rate was significantly lower in patients with MNZ resistance, AMPC+MNZ resistance, CAM+MNZ resistance, and in patients resistant to all three types. This suggests that MNZ resistance has a significant impact on the success rate of *H. pylori* eradication and should be considered in future eradication strategies.

Keywords: *Helicobacter pylori*; eradication; multiple antibiotic resistance

1. Introduction

Helicobacter pylori (*H. pylori*) is a major causative microorganism of gastritis and peptic ulcer [1–4], and it is widely recognized that long-term infection increases the risk of developing gastric cancer [5]. Therefore, *H. pylori* eradication therapy is considered one of the crucial treatment strategies in the prevention of gastric cancer and the management of gastrointestinal diseases. Proton pump inhibitors (PPIs) + amoxicillin (AMPC) + clarithromycin (CAM) have long been recommended for primary eradication in Japan, and secondary eradication with PPIs + AMPC + metronidazole (MNZ) has been used when primary eradication has failed. However, it has been reported in recent years that *H. pylori* has become increasingly antibiotic-resistant, and the success rate of standard eradication therapy has declined [6].

In particular, the increase in CAM resistance has become a serious problem in Japan, and the success rate of primary eradication of CAM-resistant *H. pylori* is reported to be about 60% [7]. The rate of resistance to MNZ, which is used for secondary eradication, is also increasing, and MNZ resistance is an important factor that decreases the success rate of eradication [8]. Furthermore, resistance to AMPC, which is used for both primary and secondary eradication, has been considered relatively infrequent, but its occurrence has been reported, and its combination with other antibiotic resistance may significantly increase the difficulty of eradication [9]. In other words, the difficulty of eradicating *H. pylori* may be further exacerbated by the increase in multidrug-resistant bacteria, which are resistant to multiple antibiotics as well as single-drug resistance [10].

In recent years, eradication therapy using vonoprazan (VPZ) instead of PPIs as a novel eradication strategy has attracted much attention, especially for its efficacy against resistant bacteria [11]. The consistent and potent action of drugs that inhibit acid secretion increases the stability and

bioavailability of acid-sensitive antimicrobial agents such as CAM and AMPC by preventing their degradation in the stomach, thereby increasing their concentration in the gastric mucosa [12–14]. VPZ has a more sustained and potent acid suppression effect compared to PPIs and may reduce the effects of CAM and AMPC resistance to some extent, as it is believed to be more likely to maintain antibiotic activity [15,16]. However, the efficacy of VPZ-based eradication therapy against multidrug-resistant *H. pylori* has not been fully established.

In this study, we conducted susceptibility testing of AMPC, CAM, and MNZ in patients who visited a university hospital for *H. pylori* eradication, and analyzed the association between resistance to each antibacterial agent and difficulty of eradication. In particular, we aimed to examine the influence of cases with resistance to two or more antimicrobial agents in addition to single agent resistance, and to determine the effect of the combination of resistant organisms on the success rate of eradication.

2. Materials & Methods

2.1. Subjects and Study Procedure

Two hundred and fifty patients who visited the outpatient clinic specializing in *H. pylori* at Hamamatsu University Hospital between April 2017 and December 2020, and who underwent *H. pylori* eradication, were included in the study. Patients were informed in writing of the purpose of the study, and completed questionnaires were obtained from patients who gave their consent.

Questions included age, gender, alcohol consumption, smoking, medication use, previous sterilization experience, and number of sterilizations performed to date. In addition, among these patients, those who had undergone antimicrobial susceptibility testing were selected.

Amoxicillin, clarithromycin, and vonoprazan were used for primary eradication, and amoxicillin, metronidazole, and vonoprazan for secondary eradication. The choice of treatment after tertiary eradication was left to the discretion of the physician, but in many cases sitafloxacin and minocycline were used instead of amoxicillin.

2.2. Susceptibility Test and Resistance Classification

In susceptibility testing, MICs (minimum inhibitory concentration) of AMPC, CAM, and MNZ were measured against the collected *H. pylori* strains, and susceptibility or resistance was determined. The criteria for resistance are as follows (AMPC: MIC > 0.03 µg/mL, CAM: MIC > 1.0 µg/mL, MNZ: MIC > 8.0 µg/mL).

Resistance patterns are those resistant to at least one organism (at least resistant to AMPC, at least resistant to CAM, at least resistant to MTZ), those resistant to at least two organisms (at least resistant to AMPC+CAM, at least resistant to AMPC+MNZ, at least resistant to CAM+MNZ), and those resistant to all three drugs (AMPC+CAM+MNZ resistant).

2.3. Statistical Analysis

Simple tabulations were performed for attributes such as gender, age, and number of *H. pylori* eradications. Next, antibiotic susceptibility of patients was tabulated by MIC to determine the presence or absence of susceptibility. Patient resistance to antibiotics was also tabulated by pattern.

The objective variables were defined as the number of sterilization attempts: one (standard) and two or more, and logistic regression analysis was used to evaluate the impact of each resistance pattern on the difficulty of sterilization. In Model 1, only resistance patterns were included as explanatory variables; in Model 2, age and gender were added as covariates; and in Model 3, age, gender, alcohol drinking status, smoking status, and medication use were added. Statistical analysis was performed with JMP 13.

This study was approved by the Medical Ethics Committee of Hamamatsu University School of Medicine (No. 17-072).

3. Results

The patient demographics of this study are shown in Table 1. Ninty-one patients were included, 41 (45.1%) male and 50 (54.9%) female. Most of the patients were middle-aged and older, in their 40s to 60s. The majority of the respondents drank alcohol, but only 6.6% smoked. More than 60% of the respondents answered that they take medication. Regarding the frequency of sterilization, 28.6% had sterilized once, 28.6% twice, 6.6% three times, 4.4% four times, and 31.9% had never sterilized before, but only examined.

Table 1. Patients’ demographic characteristics.

Characteristic	n	%
Sex		
Male	41	45.1
Female	50	54.9
Age, years		
20-30	2	2.2
31-40	11	12.1
41-50	17	18.7
51-60	21	23.1
61-70	32	35.2
71-80	7	7.7
≥81	1	1.1
Medication status		
Taking medication	53	63.7
No medication	29	31.9
Unknown	4	4.4
Smoking status		
Smoking(+)	6	6.6
Smoking(-)	82	90.1
Unknown	3	3.3
Drinking status		
Drinking(+)	50	54.9
Drinking(-)	36	39.6
Unknown	5	5.5
Number of <i>H. pylori</i> eradication treatments		
Zero	29	31.9
One	26	28.6
Two	26	28.6
Three	6	6.6
Four	4	4.4

Table 2 shows the sensitivity of the patients to the antibiotics. For each antibiotic, susceptibility was determined by MIC: 61.5% of patients were relatively susceptible to AMPC, 25.3% were susceptible to CAM, and 23.0% were susceptible to MNZ.

Table 2. The sensitivity of the patients to the antibiotics.

AMPC				CAM				MNZ			
MIC(μg/ml)	n	%	Susceptibility	MIC(μg/ml)	n	%	Susceptibility	MIC(μg/ml)	n	%	Susceptibility
0.015	56	61.5	Susceptible	0.015	1	1.1	Susceptible	0.5	1	1.1	Susceptible
0.03	27	29.7	Not susceptible	0.03	13	14.3	Susceptible	2	3	3.3	Susceptible

0.06	6,	6.6	Not susceptible	0.06	9	9.9	Susceptible	4	6	6.6	Susceptible
0.12	1	1.1	Not susceptible	2	4	4.4	Not susceptible	8	11	12,1	Susceptible
0.25	1	1.1	Not susceptible	4	7	7.7	Not susceptible	16	36	39.6	Not susceptible
				8	17	18.7	Not susceptible	32	34	37.4	Not susceptible
				16	17	18.7	Not susceptible				
				32	10	11.0	Not susceptible				
				64	13	14.3	Not susceptible				

MIC: Minimum Inhibitory Concentration.

Table 3 shows the antibiotic resistance of the patients. Those resistant to at least CAM and at least MNZ exceeded 70% each. More than 60% of the patients were resistant to at least two drugs in combination with CAM and MNZ, and less than 30% were resistant to all of AMPC, CAM and MNZ.

Table 3. The resistance of the patients to the antibiotics.

Resistance Classification	n	%
Resistant to at least AMPC	34	37.4
Resistant to at least CAM	68	74.7
Resistant to at least MNZ	70	76.9
Resistant to at least both AMPC and CAM	31	34.8
Resistant to at least both AMPC and MNZ	28	30.8
Resistant to at least both CAM and MNZ	56	61.5
Resistant to all AMPC, CAM and MNZ	26	28.6

Table 4 shows the results of the logistic regression analysis of the association between antibiotic resistance patterns and difficulty of eradication. Patients who were resistant to at least AMPC and MNZ, at least CAM and MNZ, and resistant to all AMPC, CAM, and MNZ were significantly more difficult to eradicate in Models 1, 2, and 3. Patients resistant to at least MNZ were also significantly more difficult to eradicate in Models 2 and 3.

Table 4. Association between antibiotic resistance patterns and difficulty of eradication.

Model 1				Model 2			Model 3		
	OR	95%CI	P-value	OR	95%CI	P-value	OR	95%CI	P-value
Resistant to at least AMPC									
Susceptible		1 (Reference)			1 (Reference)			1 (Reference)	
Not Susceptible	2.36	0.85-6.90	0.101	2.34	0.82-6.98	0.111	2.53	0.84-7.61	0.097
Resistant to at least CAM									
Susceptible		1 (Reference)			1 (Reference)			1 (Reference)	
Not Susceptible	1.84	0.53-6.54	0.330	2.29	0.62-8.99	0.211	2.43	0.63-10.17	0.198
Resistant to at least MNZ									
Susceptible		1 (Reference)			1 (Reference)			1 (Reference)	
Not Susceptible	3.56	0.98-14.89	0.054	4.62	1.18-21.47	0.027	4.93	1.21-24.42	0.025
Resistant to at least both AMPC and CAM									
Susceptible		1 (Reference)			1 (Reference)			1 (Reference)	
Not Susceptible	1.78	0.63-5.20	0.277	1.72	0.59-5.02	0.321	1.87	0.61-5.70	0.272

Resistant to at least both AMPC and MNZ									
Susceptible		1 (Reference)			1 (Reference)			1 (Reference)	
Not Susceptible	3.33	1.09-10.24	0.036	3.80	1.23-13.21	0.019	4.61	1.39-18.29	0.012
Resistant to at least both CAM and MNZ									
Susceptible		1 (Reference)			1 (Reference)			1 (Reference)	
Not Susceptible	4.14	1.38-13.43	0.011	5.42	1.67-20.35	0.004	5.71	1.72-21.99	0.004
Resistant to all AMPC, CAM and MNZ									
Susceptible		1 (Reference)			1 (Reference)			1 (Reference)	
Not Susceptible	2.98	1.01-9.76	0.049	3.23	1.06-11.03	0.039	3.80	1.17-14.41	0.026

Analysis method: Logistic regression analysis. Notes: Model 2 was adjusted for age and sex. Model 3 was adjusted for age, sex, smoking status, drinking status and medication status. Bold text, statistically significant at P<0.05. Abbreviations: OR, odds ratio; CI, confidence interval.

4. Discussion

In this study, we examined the association between antimicrobial resistance of *H. pylori* and difficulty of eradication, and found a trend toward lower eradication success rates, at least in cases with MNZ resistance The magnitude of the impact of MNZ resistance on eradication reflects the fact that MNZ plays a major role in secondary eradication in Japan The magnitude of the effect of MNZ resistance on eradication is thought to reflect the major role of MNZ in secondary eradication in Japan. It was also suggested that the presence of multidrug resistance (especially AMPC+MNZ resistance and CAM+MNZ resistance) may further reduce the success rate of eradication.

On the other hand, the number of CAM-resistant bacteria has traditionally also increased significantly, and is considered to be a major factor in the decline in the success rate of eradication therapy [17]. In particular, the high rate of resistance to CAM used for primary eradication in Japan makes it likely that a relatively large number of patients will be transferred to secondary eradication [18], and the increase in MNZ resistance is a serious clinical problem [19]. Furthermore, results suggest that multidrug resistance with CAM and MNZ together increases the difficulty of eradication. However, the effect of AMPC+CAM resistance was limited in part because MNZ, used in secondary eradication, was still effective, albeit at an increased resistance rate. It is also possible that the impact of AMPC+CAM resistance was not statistically evident because the distribution of AMPC resistance was lower than that of CAM resistance.

The strength of this study lies in the detailed analysis of multiple resistance patterns and the comprehensive evaluation of the impact of each on the eradication success rate. In the method, the objective variables were defined as the number of sterilizations once (standard) and two or more times, and logistic regression analysis was used to evaluate the impact of each resistance pattern on the difficulty of sterilization. The reason why the objective variable was set as the criterion for the number of times of sterilization (1 time) was that patients who did not receive sterilization may have specific backgrounds (presence of symptoms, practice policy, etc.), and we judged that excluding patients who had not received sterilization would allow clearer analysis. Furthermore, by analyzing *H. pylori* resistance to AMPC, CAM, and MNZ as “resistant to at least ____,” we believe we were able to reflect more realistically the impact of resistant bacteria in actual clinical practice. In addition, the use of patient data from a specialized outpatient clinic at a university hospital revealed the actual situation of resistant bacteria, which are difficult to detect in general clinics. Furthermore, the multivariate analysis using logistic regression analysis made it possible to take into account the background factors of patients, suggesting that the analysis provided useful information for clinical decision making.

On the other hand, this study has several limitations. First, the patients included in the study were limited to a single institution, which does not necessarily reflect the distribution of resistant strains in different regions. In addition, it is a retrospective observational study, which limits the estimation of causal relationships. Furthermore, the genetic background and detailed molecular mechanisms of the resistant bacteria have not been examined, and more detailed analysis is required

in future studies. In the future, more generalizable data should be accumulated through multicenter collaborative and prospective studies, and the effects of resistant bacteria should be analyzed more precisely.

5. Conclusions

Difficulty in eradicating *H. pylori* was closely associated with an increase in multidrug-resistant organisms. The impact of MNZ resistance was particularly pronounced, highlighting the need for new treatment strategies. These results strongly suggest that conventional eradication therapy needs to be revised as the number of resistant bacteria increases. In particular, the proper use of antibiotics and monitoring of resistant bacteria is urgently needed to establish new treatment strategies.

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Conflicts of Interest: The authors declare no conflict of interest.

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