Eradication therapy for Helicobacter pylori infection based on the antimicrobial susceptibility test in children: A single-center study over 12 years

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Title: Eradication therapy for *Helicobacter pylori* infection based on the antimicrobial susceptibility test in children: A single-center study over 12 years

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Miyata E collected material and clinical data from patients.

Miyata E and Kudo T analyzed and interpreted the data.

Miyata E wrote the manuscript, and Kudo T, Ikuse T, and Shimizu T revised the manuscript.

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All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of this work were appropriately investigated and resolved.

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Abstract

Background: *Helicobacter pylori* (*H. pylori*) infection causes chronic gastritis, duodenal and to a lesser extent, gastric ulcers, and gastric cancer. Most *H. pylori* infections are acquired in childhood, and effective treatment of childhood infection is very important. Esophagogastroduodenoscopy (EGD) is useful for endoscopic diagnosis, mucosal tissue biopsy, and culture examination for *H. pylori* in children and adults. In this paper, we report results of susceptibility tests and eradication rates in *H. pylori* positive children who underwent EGD over a 12-year period.

Materials and Methods: The subjects were *H. pylori* positive pediatric patients who had gastrointestinal symptoms and underwent EGD in the Department of Pediatrics, Juntendo University Hospital (January 2007–December 2018). Patients underwent serum IgG antibody tests, fecal antigen tests, or urea breath tests, and subsequently, culture tests by gastric mucosal biopsy during EGD. *H. pylori* positivity was defined as a positive result on both tests. Patients received triple therapy for 14 days using our regimen, and eradication was assessed at 2, 6, and 12 months after therapy.

Results: Forty-five patients were *H. pylori* positive, and the overall clarithromycin (CAM) resistance rate was 71.1 % (32/45). The CAM resistance rate for the 2013–2018 period was significantly higher than the 2007–2012 period (52.6% vs. 84.6%, $p<0.05$).

According to the results of the antimicrobial susceptibility test, we prescribed effective antibiotics, and this resulted in a primary eradication rate of 97.7%.

Conclusions: We suggest that antimicrobial susceptibility testing can significantly improve rates of primary eradication of *H. pylori* infection.

Introduction

Helicobacter pylori (*H.pylori*) is a gram-negative spiral bacillus, and *H. pylori* infection causes chronic gastritis, duodenal and to a lesser extent, gastric ulcers, gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric cancer. It has been reported that about 35% of Japanese adult people have had an *H. pylori* infection and about 1.8% of children aged 0–11 years[1]. The majority of *H. pylori* infections can occur in childhood. Since effective treatment during childhood is very important, the diagnosis and treatment of *H. pylori* infection in childhood has been gaining recent attention.

Esophagogastroduodenoscopy (EGD) is useful for endoscopic diagnosis, mucosal tissue biopsy, and culture examination for *H. pylori* in pediatric patients as well as adults. We have been working on *H. pylori* eradication therapy based on the antimicrobial susceptibility test for the culture of *H. pylori* using a gastric mucosal biopsy obtained during EGD for more than 10 years. In this study, we report *H. pylori* positivity rates in children who undergo diagnostic EGD and *H. pylori* tests, as well as the results of susceptibility tests and eradication rates over a 12-year period.

Methods

Patients

The subjects included *H. pylori* positive pediatric patients who underwent EGD in the Department of Pediatrics at Juntendo University Hospital from January 2007 to December 2018. This study was reviewed and approved by Juntendo University Hospital Ethics Committee (No. 19-211).

Diagnosis of H. pylori infection

We performed an initial serum *H. pylori* IgG antibody test, a fecal *H. pylori* antigen test, or a urea breath test, and subsequently a culture test by gastric mucosal biopsy (the body and antrum of the stomach) during EGD for children who had symptoms associated with *H. pylori* infection. *H. pylori* positivity was defined by a positive result on both the *H. pylori* test and culture test.

Antimicrobial susceptibility tests and eradication therapy

All patients and/or their parents provided informed consent after receiving information about treatment and side effects before the eradication therapy. We

performed antimicrobial susceptibility tests using biopsies from the body and antrum of the stomach, and resistance was defined as a minimum inhibitory concentration (MIC) over 1 μg/mL for CAM, MIC over 0.06 μg/mL for AMPC, and MIC over 8 μg/mL for MNZ. All antimicrobial susceptibility tests for *H.pylori* used both agar plate dilution methods and e-test with biopsy tissue in the inspection facility.

We performed triple therapy as a first eradication attempt for 14 days using two antibiotics from the following list: clarithromycin (CAM), amoxicillin (AMPC), metronidazole (MNZ), and minomycin (MINO), along with proton pump inhibitors (PPIs). The dose of CAM was 20 mg/kg/day (>40 kg 800 mg/day), AMPC was 50 mg/kg/day (>40 kg 1500 mg/day), MNZ was 10-20 mg/kg/day (>40 kg 1000 mg/day) and MINO was 4 mg/kg/day (>40 kg 200 mg/day). The PPIs used were lansoprazole (1.5 mg/kg/day, >20 kg 30 mg/day), omeprazole (1.0 mg/kg/day, >40 kg 40 mg/day), or rabeprazole (0.5 mg/kg/day, >40 kg 20 mg/kg/day). We selected antibiotics with reference to the results of antimicrobial susceptibility tests. We used PPI + AMPC + CAM (PAC regimen) for CAM sensitive patients, PPI + AMPC + MNZ (PAM regimen) for CAM alone resistant patients, and PPI + MNZ + MINO for CAM and AMPC resistant or CAM, AMPC, and MNZ resistant patients. All patients took a single probiotic agent (0.1g/kg/day, >30kg 3g/day) orally with the eradication therapy to prevent severe diarrhea due to antibiotics.

Judgement of eradication

The judgement of eradication was performed 2, 6, and 12 months after eradication therapy using a urea breath test or stool *H. pylori* antigen test.

Statistical analysis

We used Microsoft Excel (Microsoft, Redmond WA, USA) for statistical analysis, a Mann-Whitney-U test for comparison between the two groups, and $p<0.05$ was regarded as statistically significant.

Results

A total of 575 EGD examinations were performed on 455 patients with gastrointestinal symptoms. An *H. pylori* test was performed on 119 patients and 45 patients (37.8%) were *H. pylori* positive (Figure 1).

H. pylori positive patients

The male-to-female ratio of *H. pylori* positive patients was 24:21, and the mean age at the time of EGD was 12.0 ± 2.8 years (range: 5.1–15.9 years). The chief complaint

was abdominal pain in 27 patients (60.0%), tarry and bloody stools in six patients (13.3%), positive *H. pylori* test in six patients (13.3%), idiopathic thrombocytopenic purpura in two patients (4.4%), nausea in two patients (4.4%), and anemia in two patients (4.4%). EGD findings included nodular gastritis in 41 patients (91.1%), gastric ulcers in two patients (4.4%), and duodenal ulcers in nine patients (20.0%) (Table 1). Four patients had gastric atrophy of C-1 in the endoscopic Kimura-Takemoto classification. None of the patients had a previous history of eradication therapy for *H. pylori*.

Antimicrobial susceptibility tests and eradication therapy

The antimicrobial tests showed 32 patients (71.1%) had some drug resistance, resistance to CAM alone in 26 patients (57.8%), resistance to CAM and AMPC in four patients (8.9%), resistance to CAM and MNZ in one patient (2.2%), and resistance to CAM, AMPC, and MNZ in one patient (2.2%) (Table 2). Thirteen patients received the PAC regimen, and 26 patients received the PAM regimen (Table 2). Lansoprazole was used in 37 patients, rabeprazole was used in 7 patients, and omeprazole was used in one patient. The overall primary CAM resistance rate was 71.1% (32/45), and there were no patients with resistance to AMPC alone. The CAM resistance in the primary and secondary treatment was 71.7% (33/46). There was no difference between agar plate

dilution methods and e-test.

We divided the 12-year period into two halves—the first half and the second half—to compare the CAM resistance rate. The CAM resistance rate in the second period (2013-2018) was significantly higher than that in the first period (2007-2012) (84.6% vs. 52.6%, $p<0.05$) (Figure 2).

The primary eradication probability was 97.7% excluding a patient who dropped out. All patients became negative for *H. pylori* after eradication therapy for 2 months, except for the failure case. There was no reinfection documented during the study period. Some patient details in the medical charts were unavailable; however, there was no indication in the medical records that the patients had poor adherence to the drugs prescribed during the study period.

Eradication failure case

Primary eradication therapy failed in only one *H. pylori* positive patient. This patient was a 14-year-old girl with recurrent abdominal pain who was positive for serum *H. pylori* IgG test and showed a positive urea breath test in the first assessment. Her

endoscopic finding was nodular gastritis without peptic ulcers. The antimicrobial susceptibility test showed CAM single resistance, and therefore, we performed primary eradication therapy using the PAM regimen. However, after 2 months, the urea breath test remained positive and upper abdominal pain persisted, so we judged this as eradication failure. In the EGD 6 months after the first eradication therapy, nodular gastritis remained and *H. pylori* was detected in the culture test. Because the patient was resistant to CAM and AMPC on the second antimicrobial susceptibility test, we performed secondary eradication therapy using MINO, MNZ, and vonoprazan (VPZ). After 2 months, her epigastric pain disappeared and the urea breath test was negative. Since oral compliance was good in the primary eradication therapy, and *CYP2C19* gene mutation was not observed, we suspected that the primary eradication failure had been due to insufficient suppression of acid secretion.

Adverse events

In this study, though mild diarrhea occurred in two patients, no adverse events required additional treatment due to the *H. pylori* diagnostic test, EGD, or eradication therapy, such as vomiting, severe diarrhea, skin rash, allergic reaction to antibiotics, or weight loss were detected.

Discussion

The "Guidelines for the management of *Helicobacter pylori* infection in Japan: 2016 Revised Edition" recommend drug selection based on culture tests because CAM resistance has reportedly progressed to 38.5%[2]. In this study, CAM-resistant *H. pylori* accounted for 70% of the total cases, which was higher than that reported in a 2017 national survey (43.4%) and reports from European (21.2%) in children[3,4]. We consider that this is likely due to the high prescription rate of CAM in childhood. The patients received CAM as the first-line therapy for *H.pylori*, but they might have used such antibiotics for other diseases in the past. In the pediatric field in recent years, CAM resistance for bacteria has become a problem in various fields with increased administration of CAM. In fact, the macrolides, including CAM, are more frequently used in Japan compared to in the EU[5], and one study reported that the 58.2% of antimicrobial drugs prescribed to children in Japan are macrolides[6]. The CAM resistance rates for *H.pylori* were 24.7% from 2002 to 2006, 31% from 2010 to 2011, 38.5% from 2013 to 2014, and 38.0% from 2015 to 2016 in Japanese adult surveillance[7]. Furthermore, the primary CAM resistance rate in young patients (less than 30 years old) was 57.9% from 2012 to 2013[8]. In addition, the CAM resistance rate was 52.6% in the first six years

from 2007 to 2012 and 84.6% in the second six years from 2013 to 2018 in this study. Kato et al. demonstrated that the CAM resistance rate was 32.4% from 1999 to 2002 and 40.7% from 2003 to 2007 in children who had no previous history of eradication therapy[9] (Table 3). These studies indicated that CAM resistance increased with time. Because our study was a single center study with a small sample size, there may be bias in the patients included; therefore, it is necessary to consider multicenter studies.

In a retrospective multicenter study of Japanese pediatric *H. pylori* patients, the primary eradication rate of PAC therapy was as low as 77.4%. On the other hand, there was a report that the primary eradication rate for children and young people was 93.4% when PAC therapy was applied to CAM sensitive patients and PAM therapy was applied to CAM-resistant patients based on the result of antimicrobial susceptibility tests[10]. In this study, as in the previous report, eradication therapy was performed with reference to the antimicrobial susceptibility results. Therefore, this may have accounted for the extremely high primary eradication rate (97.7%) (Table 4). Several reports have suggested that tailoring therapy by selecting the eradication drugs after antimicrobial susceptibility tests can raise the eradication rate, and the "Guidelines for the management of *Helicobacter pylori* infection in Japanese children" published in 2018

and guidelines from ESPGHAN recommend drug selection based on antimicrobial susceptibility results[11,12,13].

The rate of AMPC-resistant *H. pylori* was 0% in 2010 according to the report by Kato et al.[8], but the nationwide survey by Okuda et al. showed that the rate of AMPC-resistant *H. pylori* was 9.2% from 2006 to2013[3]. The national survey included primary and secondary resistance for *H.pylori*. In this study, the rate of AMPC single resistance was 0%, and the rate of multidrug resistance was 11.1%. In addition, the rate of AMPC resistance, as well as CAM resistance, tended to increase with time. We considered that this result also related to the prescription of antibiotics in childhood.

In *H. pylori* eradication therapy, suppression of gastric acid secretion is important. In a study comparing the antacid effects of VPZ and PPI, it was reported that VPZ had a longer antacid effect than PPI[14]. In addition, triple combination therapy with VPZ showed significantly higher primary eradication rates compared to lansoprazole in Japanese clinical trials in adults[15]. Therefore, administration of VPZ might play a role in primary eradication as well as PPI. In the failed patient in this study, VPZ was used for the secondary eradication given the possibility of insufficient acid secretion

suppression in the primary eradication. One study reported *H. pylori* eradication using potassium ion competitive acid blockers (P-CABs) for *H. pylori* positive junior high school students[16], but in the pediatric field, the safety of VPZ administration has not been established, and more clinical trials are required in the future.

This study had several limitations. First, this study was a single-center study, and our hospital was a facility that receives referral patients; therefore, regional differences may exist. As our policy, we perform an EGD and *H. pylori* tests, if we suspect a digestive disease. All patients had some abdominal symptoms and underwent EGD. This could lead to sampling biases. Second, this study was a retrospective study. Although we considered that the higher CAM resistance was associated with a history of antibiotic use, we could not investigate medication history in most patients.

In summary, we correctly diagnosed *H. pylori* infection in children using non-invasive tests and biopsy tests using EGD. We performed eradication therapy with reference to the antimicrobial susceptibility results of this study, thereby allowing us to obtain the extremely high primary eradication rate.

Conclusions

Over the past two decades, due to the increased administration of CAM for many types of infections, rates of CAM resistance have increased. Despite this, an extremely high rate of *H. pylori* eradication was obtained at our department using EGD and the antimicrobial susceptibility test from the *H. pylori* culture of gastric mucosal biopsies to guide the choice of antibiotic therapy. We recommend using an antimicrobial susceptibility test prior to treatment to increase the rates of primary eradication in *H. pylori* positive children.

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Figure legends

Figure 1. The diagnosis of *H. pylori* infection and eradication therapy in our hospital

A patient was defined by both a positive test result (serum *H. pylori* IgG antibody test, fecal *H. pylori* antigen test, or urea breath test) and a positive culture test. Eradication therapy was performed for 14 days based on antimicrobial susceptibility test. Eradication was assessed 2, 6, and 12 months after EGD using a stool antigen test or urea breath test.

H. pylori, *Helicobacter pylori*; EGD, esophagogastroduodenoscopy; CAM, clarithromycin

Figure 2. CAM resistance rates from 2007 to 2018

The CAM resistance rate shows an increase with time.

CAM, clarithromycin

Table 1. The characteristics of *H. pylori* positive patients

		H. pylori positive patients (n = 45)
Age (years), mean ± SD (range)		12.0 ± 2.8 (5.1–15.9)
Sex (Male/Female)		24 / 21
Chief complaint, n (%)	Abdominal pain	27 (60.0%)
	Tarry and bloody stool	6 (13.3%)
	Positive Hp test	6 (13.3%)
	Idiopathic thrombocytopenic	2 (4.4%)
	Nausea	2 (4.4%)
	Anemia	2 (4.4%)
EGD findings	Nodular gastritis	41 (91.1%)
	Gastric ulcers	2 (4.4%)
	Duodenal ulcers	9 (20.0%)

H. pylori, *Helicobacter pylori*; SD, standard deviation; EGD, esophagogastroduodenoscopy

Table 2. The results of antimicrobial susceptibility tests in *H. pylori* positive children and the eradication regimen

Results of antimicrobial susceptibility tests	n		Eradication regimen
No resistance	13	(28.9%)	PPI+AMPC+CAM
Total CAM resistance	32	(71.1%)	
CAM single resistance	26	(57.8%)	PPI+AMPC+MNZ
CAM and AMPC resistance	4	(8.9%)	PPI+MNZ+MINO
CAM and MNZ resistance	1	(2.2%)	PPI+AMPC+MINO
CAM, AMPC, and MNZ resistance	1	(2.2%)	PPI+MNZ+MINO

H. pylori, *Helicobacter pylori*; CAM, clarithromycin; AMPC, amoxicillin; MNZ, metronidazole; MINO, minomycin, PPI, proton pump inhibitors

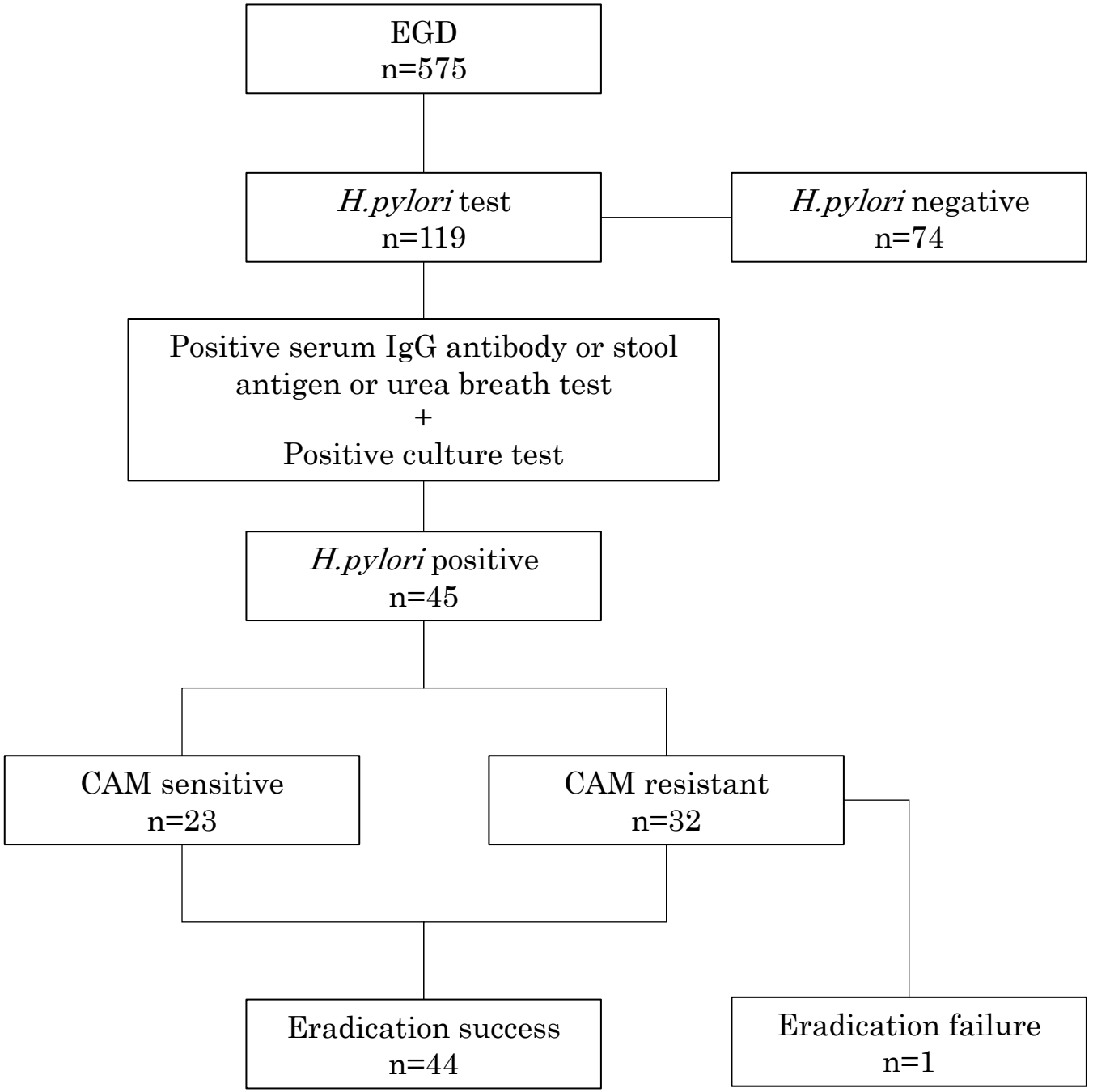
Table 3. The report of drug resistance rates in *H. pylori* positive children

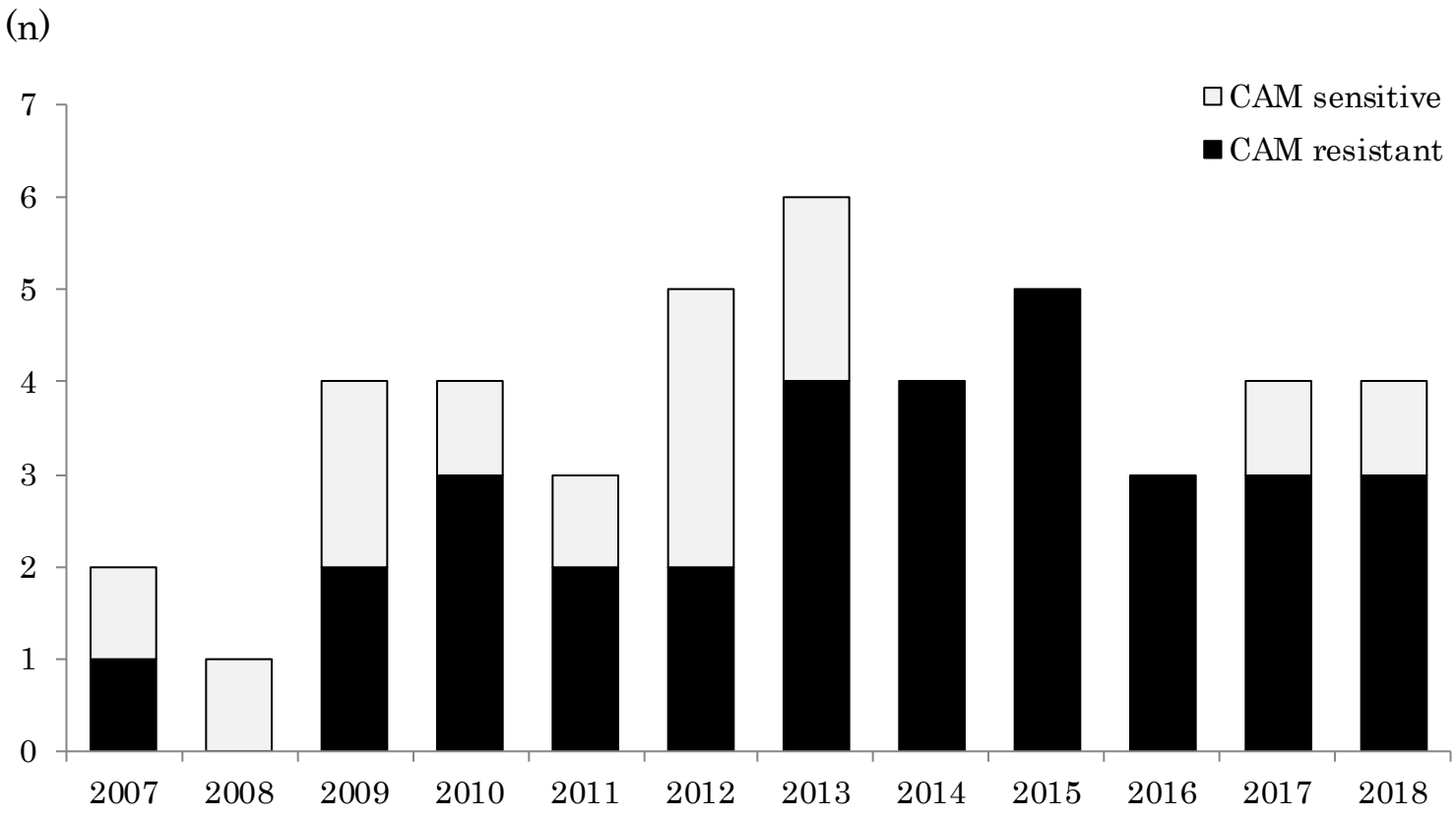
	Investigation period	n	Age range (year)	CAM resistance rate (%)	AMPC resistance rate (%)
Kato et al., 2010[9]	overall	61	4-18	36.1	0
	1999-2002	34		32.4	0
	2003-2007	27		40.7	0
Our study	overall	45	5-15	71.1	11.1
	2007-2012	19		52.6	5.3
	2013-2018	26		84.6	15.4

CAM, clarithromycin; AMPC, amoxicillin

Table 4. The reports of primary eradication probability in *H. pylori* positive children

	Investigation period	n	primary eradication probability (%)
Kato et al., 2010[9]	1999-2007	61	85.7
Okamura et al., 2014[8]	2000-2013	88	94.3
Okuda et al., 2017[3]	1997-2013	320	73.1
Our study	2007-2018	45	97.7


EGD
n=575
H.pylori test
n=119
H.pylori negative
n=74
Positive serum IgG antibody or stool antigen or urea breath test
+
Positive culture test
H.pylori positive
n=45
CAM sensitive
n=23
CAM resistant
n=32
Eradication success
n=44
Eradication failure
n=1


(n)
CAM sensitive
CAM resistant
7
6
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2
1
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2007
2008
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2018

n, number