Research Article

Clinical Evaluation of a Novel Stool Antigen Test Using Bioluminescent Enzyme Immunoassay for Detecting Helicobacter pylori

Toshihiko Kakiuchi,1 Muneaki Matsuo,1 Yasuhisa Sakata,2 and Kazuma Fujimoto3

1Department of Pediatrics, Faculty of Medicine, Saga University, Saga 849-8501, Japan
2Department of Internal Medicine, Faculty of Medicine, Saga University, Saga 849-8501, Japan
3Department of Gastroenterology, International University of Health and Welfare, Ohkawa, Fukuoka 831-0016, Japan

Correspondence should be addressed to Toshihiko Kakiuchi; kakiuchtt@cc.saga-u.ac.jp

Received 8 February 2022; Accepted 7 April 2022; Published 21 April 2022

1.Introduction

One of the major risk factors for gastric cancer is Helicobacter pylori (H. pylori) infection [1–4]. The risk of stomach cancer among individuals who are not infected with H. pylori is extremely low [5, 6], and its risk is lowered by the eradication therapy for H. pylori. In addition, preventing gastric cancer is more possible during the early stage of bacterial infection [7–10].

In Japan, the gastric cancer mortality rate is second highest in males and third highest in females among all cancer mortalities [11]. Recently, the number of municipalities in Japan that conduct H. pylori testing and eradication in middle school students to prevent gastric cancer has been increasing [12, 13]. In 2016, Saga Prefecture became one of the first prefectures in Japan to begin H. pylori testing in all third-year middle school students and eradication therapy for those who tested positive [14]. H. pylori tests that were conducted throughout Japan differ across municipalities; many regions use the urine H. pylori antibody test as the primary test and then use either the urea breath test or the stool H. pylori antigen test as a secondary test [15–17]. A
kidney disease screening program was established for school-age children in Japan; thus, urine is used for the primary test. The fecal antigen test has been used as an examination method for *H. pylori* infection as the secondary test, thus its high inspection accuracy is required compared with primary test methods.

BLEIA™ “EIKEN” *H. pylori* antigen (B[EIA]; Eiken chemical CO., LTD., Tokyo, Japan) that is based on the bioluminescent enzyme immunoassay (BLEIA) method [18] was newly developed for detecting *H. pylori* antigen in feces with high sensitivity. B (EIA) applies firefly luciferase luminescence, which is a type of bioluminescence, wherein the substrate luciferin is converted to oxyluciferin by the firefly luciferase in the presence of adenosine triphosphate, magnesium ion, and dissolved oxygen, thus light emission is obtained. The enzyme stability has improved using heat-resistant biotinylated luciferase [19, 20].

This study aimed to clinically evaluate the efficacy of B (EIA) for school-age children and compare it with that of the already commercially available rapid test kits.

2. Methods

2.1. Ethical Approval and Consent to Participate. The institutional review board of Saga University Hospital approved the present study (approval numbers: 2019-04-04). The study methodology was explained before obtaining written informed consent from all participants and their parents or guardians.

2.2. Enrollment. The longitudinal project for *H. pylori* screening and treatment among junior high school third-grade students in Saga Prefecture started in 2016, which aimed to prevent primary gastric cancer [14]. Figure 1 shows a flowchart of the junior high school third-grade students in Saga Prefecture in 2019. Among 8,216 junior high school students aged 14 or 15 years, 7,512 received a screening test (RAPIRAN™; Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) to detect anti-*H. pylori* immunoglobulin-G antibody by immunochromatography. A screening program for kidney diseases was established in Saga Prefecture, which targets third-grade students in junior high school. Given the full inclusivity of students during this test through simple urine examination, the established system was used to obtain urine samples to screen for *H. pylori* infection. A total of 7,325 students tested negative for *H. pylori* with the urinary test. Among 187 students who were tested positive in the screening urinary test underwent an *H. pylori* stool antigen test (SAT) (Quick Chaser™ *H. pylori* [Q (IC)]; Mizuho Medy Co., Ltd., Tosu City, Saga, Japan). The following are the exclusion criteria for this study: (i) students who had taken medications, including proton pump inhibitors (PPIs), H2 receptor antagonists, antacids, probiotics, mucosal protective agents, and antibiotics within 6 months before enrollment; (ii) students who had outpatient hospital visits because of sickness; (iii) students with chronic gastrointestinal diseases, such as functional gastrointestinal disorders, celiac disease, eosinophilic gastrointestinal disorder, inflammatory bowel diseases, etc.; and (iv) students who had undergone eradication therapy for *H. pylori*.

2.3. B (EIA) vs. Q (IC). A fecal *H. pylori* antigen test was performed in 141 students as a secondary screening using Q (IC) in our program. Q (IC) was measured following the methods described in the manufacturer’s instructions. B (EIA) was measured with a fully automatic biochemical luminescence immunoassay device (BLEIA™-1200) and a BL Sampling Bottle (Eiken Chemical Co., Ltd., Tokyo, Japan). The measurement was carried out by installing BL Sampling Bottle on the measuring device on which the reagent was already installed. The measurement data were obtained as a cutoff index (COI), which is a value obtained by dividing the amount of light emitted from each sample by the cutoff value calculated from the amount of light emitted by the calibrator. A COI of 1.0 or higher was considered positive and a COI of <1.0 was considered negative.

2.4. Comparison of Multiple Fecal Antigen Test Kits. A comparative test was conducted between B (EIA) and extracorporeal diagnostic agents that were marketed in Japan as of 2019. The comparison target tests were Q (IC), Testmate pylori antigen EIA (T[EIA]); Hitachi Kasei Diagnostics Systems Co., Ltd., Tokyo, Japan), Testmate rapid pylori antigen (T[IC]; Nippon Becton Dickinson Co., Ltd., Tokyo, Japan), Meridian HpSA ELISA II (M[EIA]; Pacific Bridge Medical Co., Ltd., Tokyo, Japan), and Immunocard STAT HpSA (II[IC]; Pacific Bridge Medical Co., Ltd., Tokyo, Japan). Measurements by Q (IC), T (EIA), T (IC), M (EIA), and I (IC) were performed according to the manufacturer’s instructions. The equipment used for the measurement was BLEIA™-1200 for B (EIA) based on the BLEIA method, and an immunochromatographic reader C10066 (Hamamatsu Photonics Co., Ltd., Hamamatsu, Shizuoka) for Q (IC), T (IC), and I (IC) based on the immunochromatography method. T (EIA) and M (EIA) based on the EIA method used plate readers Infinite 200 PRO M Plex (Tekan Japan Co., Ltd., Kawasaki, Kanagawa). In addition, the reagent based on the immunochromatography method was visually evaluated using the comprehensive determination of the immunochromatographic reader.

2.5. The Detection Performance of *H. pylori* ATCC43504 Standard Strain. The sample to confirm the detection performance was prepared by diluting 1.5 mg/mL of *H. pylori* antigen (ATCC43504 strain) with a buffer solution in a stool collection container that is dedicated to each reagent of 10–20,000 pg/mL.

2.6. The Detection Performance of *H. pylori* Antigen in Commercial Human Fecal Specimens. Commercially available *H. pylori* antigen-positive human feces (Discovery Life Sciences, Inc) were collected and suspended in stool collection containers that are dedicated to each test kit, and the obtained stool suspensions were diluted with the buffer solution of each company’s stool collection containers. A
total of 5 types of *H. pylori* antigen-positive human feces and 5 types of *H. pylori* antigen-negative human feces were prepared.

### 3. Results

#### 3.1. B (EIA) vs. Q (IC).

Table 1 shows the correlation test results using Q (IC) and B (EIA) in 141 participants. The positive and negative concordance ratios of B (EIA) to Q (IC) were 100.0% (110/110) and 71.0% (22/31), respectively. The overall concordance ratio was 93.6% (132/141). A comparative study of B (EIA) and Q (IC) confirmed 9 divergent samples. A dilution linearity test was conducted in 9 cases to estimate the cause of result divergence. The dilution test sample of the dissociated sample was prepared by diluting the stool suspension that was collected and suspended in BL Sampling Bottle 2 to 16 times with the buffer solution of BL Sampling Bottle. As a result, all samples almost showed linearity, without the characteristics seen in the nonspecific reaction (Figure 2(a)). *H. pylori* antigen cutoff values for 9 samples without dilution ranged from 1.3 to 87.4 on B (EIA) (Figure 2(b)).

#### 3.2. The Detection Performance of *H. pylori* Antigen of ATCC43504 Standard Strain.

The measurement of *H. pylori* antigen dilution series of 10–20,000 pg/mL that was prepared by diluting 1.5 mg/mL of *H. pylori* antigen (ATCC43504 standard strain) resulted in positive ranges of B (EIA) as 39 pg/mL or more; Q (IC) as 2,500 pg/mL or more; T (EIA), T (IC), and M (EIA) as 10,000 pg/mL or more; and I (IC) as 20,000 pg/mL or more (Table 2).

#### 3.3. The Detection Performance of *H. pylori* Antigen in Commercial Human Fecal Specimens.

The measurement of the dilution series of the suspension of 5 commercially available *H. pylori* antigen-positive human fecal samples resulted in 128–3072 dilution times in B (EIA), 8–48 times in Q (IC), 4–48 times in T (EIA), 2–48 times in T (IC), 8–192 times in M (EIA), and 4–96 times in I (IC), which were positive (Table 3). The measurement of 5 commercially available *H. pylori* antigen-negative fecal samples was negative in all reagents (Supplementary Table 1).

### 4. Discussion

B (EIA) can be positive for *H. pylori* antigen of standard strain even in samples that are diluted for 64–512 times more than the other reagents and positive for *H. pylori* antigen in commercial human fecal specimens even in samples that are diluted for 16–128 times more than the other reagents. Therefore, B (EIA) is suggested to be the reagent with the highest detection performance of *H. pylori* antigen compared with other reagents.

A comparison between the newly developed B (EIA) and Q (IC) showed a positive concordance rate of 100%, the negative concordance rate of 71.0%, and overall concordance rate of 93.6%, which was considered a favorable result. Nine dissociated specimens that were negative for Q (IC) and positive for B (EIA) were confirmed. All dilution tests of these 9 cases almost showed linearity, without characteristics
Figure 2: Dilution tests of 9 dissociated samples between B (EIA) and Q (IC). All samples almost showed linearity, without the characteristics seen in the nonspecific reaction (a). *Helicobacter pylori* antigen cutoff values for 9 samples without dilution ranged from 1.3 to 87.4 on (b) (EIA). Hp Ag, *Helicobacter pylori* antigen; COI, cutoff index.
of nonspecific reactions. Additionally, the measured value of B (EIA) of the dissociation samples was 1.3–87.4 COI (Figure 2(a)) in the range that can be evaluated as negative by other fecal H. pylori antigen test kits (Table 2); thus, all dissociation samples were H. pylori antigen-positive cases, and finally the cause of the result divergence was presumed to be false negative due to insufficient Q (IC) sensitivity.

The guidelines for H. pylori infection management in Japan suggested that the SAT has great diagnostic performance, with a sensitivity of 96%–100% and specificity of 97%–100% before eradication [21]. Diagnostic performances of different SATs are heterogeneous, which may relate to the designs of tests like EIA and immunochromatographic assay (ICA) and for the selection of antibodies, such as monoclonal and polyclonal antibodies [22]. Many studies were conducted on the performance evaluations of SAT kits [23–27], but this is the first study that simultaneously evaluated six types of SAT kits. EIA provides more reliable results than ICA [28]. In the present study, B (EIA) was considered to have better sensitivity than the EIA and IC method for H. pylori antigen.

In Japan, the number of municipalities conducting screening for H. pylori among junior high school students has increased in recent years to prevent gastric cancer [12–14, 29, 30]. H. pylori antigen testing using fecal samples is adopted because of its simplicity in screening. Moreover, it is an extremely useful examination method because of its conduction without endoscopy, and such a method is preferred in children. Attempts to extract the DNA of H. pylori bacteria and test resistance to H. pylori against clarithromycin using stool samples have been assessed [31, 32]. In the methods using feces, the fecal antigen and drug susceptibility tests can be performed using the same specimen, and the usefulness of the fecal antigen test will be further enhanced compared to UBT test. Also in this point, B (EIA) is a useful screening method to test and treat H. pylori in adolescents.

H. pylori was recognized as a pathogenic infectious agent for humans only in 1985 by Marshall and Warren [33]. The recognition of the pathogenic role of H. pylori has revolutionized medicine and gastric and duodenal pathology. This has led not only to the new description of the etiopathogenesis of some diseases of the digestive system, but also to re-thinking the prevention and therapy; for example, acute gastritis, chronic atrophic gastritis, ulcer of stomach and duodenum, mucosa-associated lymphoid tissue lymphoma, esophageal cancer, gastric adenocarcinoma, nonalcoholic steatohepatitis, etc [34]. Based on these facts, it is also the basis for test and treatment to eradicate H. pylori during adolescence to prevent gastric cancer [13, 14].

It is well established that H. pylori infection is acquired in childhood [35]. Regarding eradication therapy among children from the perspective of future gastric cancer prevention, this point remains controversial. Therefore, we still need to discover the relationships between individual and environmental factors, including incidence and pathogenesis and correlations with other systems, in order to determine when to eradicate H. pylori. At present, there is a significant reduction in the incidence of the disease worldwide and a steady decrease in the incidence of infection in childhood, probably due to better conditions of hygiene and improvement in treatment. Treatment must address compliance and antibiotic resistance during the disease [36, 37]. In Japan, cases of eradication treatment failure largely depend on the presence or absence of clarithromycin resistance [38], with CAM-resistant rate being higher in children [39]. Several studies have confirmed that probiotic supplementation is able to facilitate eradication and reduce the incidence of side effects of antibiotic therapy [34], because the microbiota can be altered by immune-mediated reactions of the organism against the bacterium [40].

The present research has limitations that need to be addressed in future studies. Study results were not compared to the gold standard urea breath test or invasive tests (bacterial culture, rapid urease test, etc.), thus positive or negative results were still controversial. The present study revealed that B (EIA) had a beneficial result in H. pylori
infection diagnosis. However, research on the eradication therapy efficacy has not been conducted. Furthermore, the effect of oral antacid administration, such as PPI, has not been evaluated. The impact of PPI is believed to be minimal using the fecal antigen test kit [41, 42]; however, this assumption was not validated. The evaluation due to the differences in the genotype of *H. pylori* was not eventually performed using B (EIA).

5. Conclusions

B (EIA) is based on the BLEIA method that applies firefly luciferase luminescence and is more sensitive than the SAT kits that are currently marketed in Japan. This test is very useful for diagnosing *H. pylori* infections, particularly in cases where noninvasive tests are preferred, such as in children.

### Table 3: Detection of *H. pylori* antigen in commercial human fecal specimens.

| Sample | Dilution rate (COI) | Measured values | Judgement |
|--------|---------------------|-----------------|-----------|
|        | B (EIA) (mAbs) | Q (IC) (mAbs) | T (EIA) (Abs) | T (IC) (Abs) | M (EIA) (mAbs) | I (IC) (mAbs) | B (EIA) | Q (IC) | T (EIA) | T (IC) | M (EIA) | I (IC) |
| Specimen 1 | ×24 | 166.4 | 29.2 | 0.243 | 14.5 | 1.143 | 8.6 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Specimen 2 | ×12 | 167.5 | 28 | 0.221 | 9.2 | 1.985 | 6 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Specimen 3 | ×12 | 130.4 | 21.6 | 0.159 | 6.5 | 0.568 | 6.5 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Specimen 4 | ×2 | 112.7 | 20.3 | 0.157 | 5.1 | 0.386 | 8.6 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Specimen 5 | ×4 | 53.2 | 12 | 0.101 | 3.5 | 0.205 | 5.2 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |

B (EIA), BLEIA™ “EIKEN” *H. pylori* antigen; Q (IC), Quick Chaser™ *H. pylori*; T (EIA), Testmate pylori antigen EIA; T (IC), Testmate rapid pylori antigen; M (EIA), Meridian HpSA ELISA II; I (IC), Immunocard STAT HpSA.
Data Availability
The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Disclosure
The funders had no role in study design, data collection, and interpretation, or the decision to submit the work for publication.

Conflicts of Interest
The authors declare that they have no conflicts of interest.

Authors’ Contributions
All the authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by TK and YS. The first draft of the manuscript was written by TK, MM and KF critically reviewed the manuscript. All the authors commented on previous versions of the manuscript and approved the final manuscript. All the measurements including the measurements with BLEIA™ “EIKEN” H. pylori antigen were performed by the corresponding author, who properly managed all test data.

Acknowledgments
The authors thank Mr. Naokazu Sasaki, Mr. Shin Ito, and Mr. Kazuhiito Sekine for their cooperation. They are also grateful to Ms. Kozue Kakiuchi and Ms. Ikumi Miyachi for providing support for the project. This study received funding from Eiken Chemical Co., LTD. (Tokyo, Japan). This study was supported by Eiken Chemical Co., Ltd. (Tokyo, Japan).

Supplementary Materials
Supplementary Table 1: Detection of H. pylori antigen negative in commercial human fecal specimens. (Supplementary Materials)

References
[1] S. Kikuchi, O. Wada, T. Nakajima et al., “Serum anti-Helicobacter pylori antibody and gastric carcinoma among young adults,” Cancer, vol. 75, no. 12, pp. 2789–2793, 1995.
[2] N. Uemura, S. Okamoto, S. Yamamoto et al., “Helicobacter pylori infection and the development of gastric cancer.” New England Journal of Medicine, vol. 345, no. 11, pp. 784–789, 2001.
[3] H. Suzuki and H. Mori, “World trends for H. pylori eradication therapy and gastric cancer prevention strategy by H. pylori test-and-treat,” Journal of Gastroenterology, vol. 53, no. 3, pp. 354–361, 2018.
[4] M. Hatakeyama, “Helicobacter pylori CagA and gastric cancer: a paradigm for hit-and-run carcinogenesis,” Cell Host & Microbe, vol. 15, no. 3, pp. 306–316, 2014.
[5] S. Ono, M. Kato, M. Suzuki et al., “Frequency of Helicobacter pylori-negative gastric cancer and gastric mucosal atrophy in a Japanese endoscopic submucosal dissection series including histological, endoscopic and serological atrophy,” Digestion, vol. 86, no. 1, pp. 59–65, 2012.
[6] T. Matsuou, M. Ito, S. Takata, S. Tanaka, M. Yoshihara, and K. Chayama, “Low prevalence of Helicobacter pylori-negative gastric cancer among Japanese,” Helicobacter, vol. 16, no. 6, pp. 415–419, 2011.
[7] W.-Q. Li, J.-Y. Zhang, J.-L. Ma et al., “Effects of Helicobacter pylori treatment and vitamin and garlic supplementation on gastric cancer incidence and mortality: follow-up of a randomized intervention trial,” BMJ, vol. 366, p. i5016, 2019.
[8] A. C. Ford, D. Forman, R. H. Hunt, Y. Yuan, and P. Mosayyed, “Helicobacter pylori eradication therapy to prevent gastric cancer in healthy asymptomatic infected individuals: systematic review and meta-analysis of randomised controlled trials,” BMJ, vol. 348, p. g3174, 2014.
[9] K. Fukase, M. Kato, S. Kikuchi et al., “Effect of eradication of Helicobacter pylori on incidence of metachronous gastric carcinoma after endoscopic resection of early gastric cancer: an open-label, randomised controlled trial,” The Lancet, vol. 372, no. 9636, pp. 392–397, 2008.
[10] S. B. Yoon, J. M. Park, C.-H. Lim, Y. K. Cho, and M.-G. Choi, “Effect of Helicobacter pylori eradication on metachronous gastric cancer after endoscopic resection of gastric tumors: a meta-analysis,” Helicobacter, vol. 19, no. 4, pp. 243–248, 2014.
[11] Y. Taniyama, K. Katanoeda, H. Chayama et al., “Estimation of lifetime cumulative incidence and mortality risk of gastric cancer,” Japanese Journal of Clinical Oncology, vol. 47, no. 11, pp. 1097–1102, 2017.
[12] T. Akamatsu, S. Ichikawa, S. Okudaira et al., “Introduction of an examination and treatment for Helicobacter pylori infection in high school health screening,” Journal of Gastroenterology, vol. 46, no. 12, pp. 1353–1360, 2011.
[13] C. Kusano, T. Gotoda, H. Ishikawa, and M. Moriyama, “The administrative project of Helicobacter pylori infection screening among junior high school students in an area of Japan with a high incidence of gastric cancer,” Gastric Cancer: Official Journal of the International Gastric Cancer Association and the Japanese Gastric Cancer Association, vol. 20, no. Suppl 1, pp. 16–19, 2017.
[14] T. Kakiuchi, M. Matsuou, H. Endo et al., “A Helicobacter pylori screening and treatment program to eliminate gastric cancer among junior high school students in Saga prefecture: a preliminary report,” Journal of Gastroenterology, vol. 54, no. 8, pp. 699–707, 2019.
[15] S. Kato, S. Fujimura, H. Udagawata et al., “Antibiotic resistance of Helicobacter pylori strains in Japanese children,” Journal of Clinical Microbiology, vol. 40, no. 2, pp. 649–653, 2002.
[16] S. Kato and S. Fujimura, “Primary antimicrobial resistance of Helicobacter pylori in children during the past 9 years,” Pediatrics International, vol. 52, no. 2, pp. 187–190, 2010.
[17] T. Akamatsu, T. Okamura, Y. Iwaya, and T. Suga, “Screening to identify and eradicate Helicobacter pylori infection in teenagers in Japan,” Gastroenterology Clinics of North America, vol. 44, no. 3, pp. 667–676, 2015.
[18] H. Ohkuma, K. Abe, Y. Kosaka, and M. Maeda, “Detection of luciferase having two kinds of luminescent colour based on optical filter procedure: application to an enzyme immunoassay,” Luminescence, vol. 15, no. 1, pp. 21–27, 2000.
[19] N. Kajiyama and E. Nakano, “Enhancement of thermostability of firefly luciferase from Luciola lateralis by a single
amino acid substitution,” *Bioscience, Biotechnology, and Biochemistry*, vol. 58, no. 6, pp. 1170–1171, 1994.

[20] H. Tatsumi, S. Fukuda, M. Kikuchi, and Y. Koyama, “Construction of biotinylated firefly luciferases using biotin acceptor peptides,” *Analytical Biochemistry*, vol. 243, no. 1, pp. 176–180, 1996.

[21] M. Kato, H. Ota, M. Okuda et al., “Guidelines for the management of *Helicobacter pylori* infection in Japan: 2016 revised edition.” *Helicobacter*, vol. 24, no. 4, Article ID e12597, 2019.

[22] E. Qiu, Z. Li, and S. Han, “Methods for detection of *Helicobacter pylori* from stool sample: current options and developments.” *Brazilian Journal of Microbiology*, vol. 52, no. 4, pp. 2057–2062, 2021.

[23] T. Kakiuchi, M. Okuda, K. Hashiguchi, I. Imamura, A. Nakayama, and M. Matsuo, “Evaluation of a novel stool antigen rapid test kit for detection of *Helicobacter pylori* infection.” *Journal of Clinical Microbiology*, vol. 57, no. 3, 2019.

[24] M. Halland, R. Haque, J. Langhorst, J. H. Boone, and W. A. Petri, “Clinical performance of the *H. pylori* quik chek and *H. pylori* CHEK assays, novel stool antigen tests for diagnosis of *Helicobacter pylori*,” *European Journal of Clinical Microbiology & Infectious Diseases*, vol. 40, no. 5, pp. 1023–1028, 2021.

[25] Y.-J. Fang, M.-J. Chen, C.-C. Chen et al., “Accuracy of rapid *Helicobacter pylori* antigen tests for the surveillance of the updated prevalence of *H. pylori* in Taiwan.” *Journal of the Formosan Medical Association*, vol. 119, no. 11, pp. 1626–1633, 2020.

[26] A. R. Opekun, C. Zierold, A. Rode et al., “Clinical performance of the automated LIAISON® meridian *H. pylori* SA stool antigen test.” *BioMed Research International*, vol. 2020, Article ID 7189519, 6 pages, 2020.

[27] H.-W. Moon, S.-Y. Lee, M. Hur, and Y.-M. Yun, “Characteristics of *Helicobacter pylori*-seropositive subjects according to the stool antigen test findings: a prospective study.” *The Korean Journal of Internal Medicine*, vol. 33, no. 5, pp. 893–901, 2018.

[28] D. S. Bordin, I. N. Voynovan, D. N. Andreev, and I. V. Maev, “Current *Helicobacter pylori* diagnostics.” *Diagnóstics*, vol. 11, no. 8, 2021.

[29] M. Okuda, T. Osaki, Y. Lin et al., “Low prevalence and incidence of *Helicobacter pylori* infection in children: a population-based study in Japan.” *Helicobacter*, vol. 20, no. 2, pp. 133–138, 2015.

[30] E. Kaji, A. Yoden, M. Otani et al., “*Helicobacter pylori* test-and-treat strategy for second-year junior high school students aimed at the prevention of gastric cancer in Takatsuki city.” *Helicobacter*, vol. 25, Article ID e12696, 2020.

[31] T. Kakiuchi, K. Hashiguchi, I. Imamura et al., “Assessment of a novel method to detect clarithromycin-resistant *Helicobacter pylori* using a stool antigen test reagent.” *BMC Gastroenterology*, vol. 20, no. 1, p. 397, 2020.

[32] T. Osaki, K. Mabe, C. Zaman et al., “Usefulness of detection of clarithromycin-resistant *Helicobacter pylori* from fecal specimens for young adults treated with eradication therapy.” *Helicobacter*, vol. 22, no. 5, 2017.

[33] B. Marshall and J. R. Warren, “Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration.” *The Lancet*, vol. 323, no. 8390, pp. 1311–1315, 1984.

[34] I. A. Charitos, D. D’Agostino, S. Topi, and I. Bottalico, “40 years of *Helicobacter pylori*: a revolution in biomedical thought.” *Gastroenterology Insights*, vol. 12, no. 2, pp. 111–135, 2021.

[35] T. Osaki, M. Konno, H. Yonezawa et al., “Analysis of intrafamilial transmission of *Helicobacter pylori* in Japanese families.” *Journal of Medical Microbiology*, vol. 64, no. Pt 1, pp. 67–73, 2015.

[36] Y.-T. Kuo, J.-M. Liou, E. M. El-Omar et al., “Primary antibiotic resistance in *Helicobacter pylori* in the Asia-Pacific region: a systematic review and meta-analysis.” *The Lancet Gastroenterology & Hepatology*, vol. 2, no. 10, pp. 707–715, 2017.

[37] T. Kakiuchi, M. Matsuo, H. Endo et al., “Gastrointestinal adverse reactions reduce the success rate of *Helicobacter pylori* eradication therapy: a multicenter prospective cohort study.” *Helicobacter*, vol. 26, no. 2, Article ID e12776, 2021.

[38] S. Kato, M. Konno, S.-i. Maisawa et al., “Results of triple eradication therapy in Japanese children: a retrospective multicenter study.” *Journal of Gastroenterology*, vol. 39, no. 9, pp. 838–843, 2004.

[39] M. Okuda, S. Kikuchi, K. Mabe et al., “Nationwide survey of *Helicobacter pylori* treatment for children and adolescents in Japan.” *Pediatrics International*, vol. 59, no. 1, pp. 57–61, 2017.

[40] T. Kakiuchi, A. Mizoe, K. Yamamoto et al., “Effect of probiotics during vonoprazan-containing triple therapy on gut microbiota in *Helicobacter pylori* infection: a randomized controlled trial.” *Helicobacter*, vol. 25, no. 3, Article ID e12690, 2020.

[41] M. Kodama, K. Murakami, T. Okimoto et al., “Influence of proton pump inhibitor treatment on *Helicobacter pylori* stool antigen test.” *World Journal of Gastroenterology*, vol. 18, no. 1, pp. 44–48, 2012.

[42] T. Shimoyama, C. Kato, M. Kodama, I. Kobayashi, and Y. Fukuda, “Applicability of a monoclonal antibody-based stool antigen test to evaluate the results of *Helicobacter pylori* eradication therapy.” *Japanese Journal of Infectious Diseases*, vol. 62, no. 3, pp. 225–227, 2009.