Microb Health Dis 2024; 6: e1108

UPDATED REVIEW ON DIAGNOSIS OF *HELICOBACTER PYLORI*

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Abstract – This review gives an overview of the main articles published in the field of *Helicobacter pylori* (*H. pylori*) diagnosis in the past year from April 2023-March 2024. The main themes include invasive and noninvasive diagnostic methods of *H. pylori,* newer methods, such as a panel of ten *H. pylori* specific antibodies test, testing for *H. pylori* using gastric fluid and outer membrane protein-cell receptor reactions diagnostic methods, and serological and pepsinogen tests. Others are Latex Agglutinin Turbidity assay and PyloPlus Urea Breath Test validations, as well as artificial intelligence cost-effectiveness. Enhanced endoscopic and histopathological diagnosis also featured Computer Assisted Diagnosis systems and *H. pylori* deep learning models. Molecular diagnostic techniques include *H. pylori* reflexive stool testing, whole genomic sequencing as well as PCR diagnostics and clarithromycin resistance testing.

Keywords: Invasive, Noninvasive, Endoscopy, Artificial intelligence, Molecular tests, Histopathology.

BACKGROUND

Considering *H. pylori* infections and its associated pathological outcomes, the diagnosis of *H. pylori* is evolving and so accurate, prompt diagnosis is therefore key to its management. These diagnostic testing can be broadly categorized into invasive and noninvasive methods of diagnosis Malfertheiner et al[1], Costa et al[2], and Sousa et al[3] (Figure 1).

METHODS

Main articles published in the field of *Helicobacter pylori* diagnosis in the past year, from April 2023 to March 2024, were screened from PubMed and Google Scholar. Search words, such as *'H. pylori* diagnosis from April 2023- March 2024', 'Diagnostic methods of *H. pylori* from April 2023-March 2024', 'Diagnosis and *H. pylori* from April 2023-March 2024', '*H. pylori* and Diagnosis from April 2023-March 2024' were used.

NON-INVASIVE METHODS

Urea Breath test (UBT) and Stool Antigen test (SAT)

The performance of the PyloPlus UBT for diagnosis of *H. pylori* infection was evaluated in comparison with the BreathTek UBT amongst pediatric patients[4]. The PyloPlus UBT performed

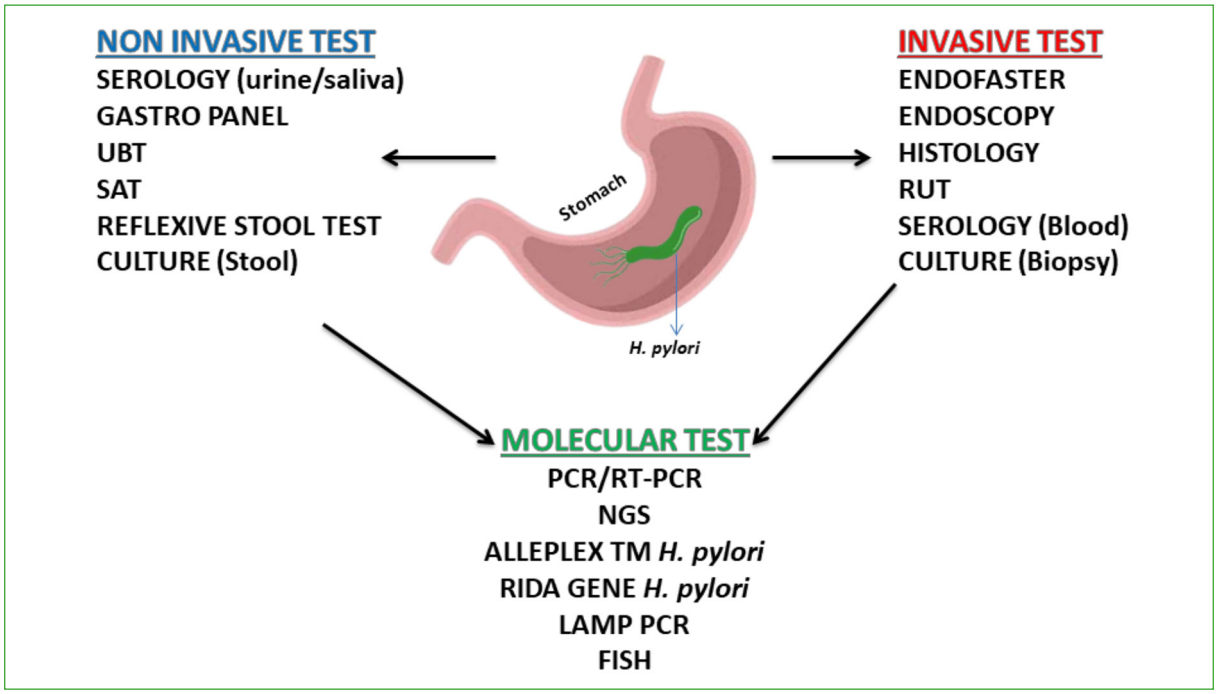

Figure 1. Invasive and non-invasive tests.

comparably with the BreathTek UBT without the need for calculation correlation factors needed for pediatric samples. Following reports from the consensus guidelines of Korea and Africa, respectively, the carbon-13 UBT and monoclonal SAT were highly accurate for the initial diagnosis and follow-up of *H. pylori* infection[5,6]. A systematic review and meta-analysis on the diagnostic accuracy for *H. pylori* diagnosis done in the elderly population showed that UBT and SAT were effective, with serology having lower specificity[7]. Shakir et al[8] suggested that testing for *H. pylori* was largely centered on SAT, UBT, and immunohistochemical staining from tissue biopsies, while a comparison of the Campylobacter-like organism (CLO) test, UBT, and Immunoglobulin G (IgG) blood test adjudged UBT as the sole diagnostic tool[9]. Biosensors, microfluidic devices, and the new bacteriophage-based *H. pylori* were also recently introduced as diagnostic tools[3].

INVASIVE METHODS OF DIAGNOSIS

Artificial intelligence and Machine Learning

Shen et al[10] evaluated the diagnostic accuracy of a computer-aided decision support system for *H. pylori* infection (CADSS-HP) based on convolutional neural network under white light imaging (WLI). CADSS-HP achieved higher sensitivity in *H. pylori* than in endoscopic diagnosis. Tang et al[11] studied a novel technique for accurately detecting *H. pylori* infection through machine learning analysis of surface-enhanced Raman scattering (SERS) spectra of gastric fluid noninvasively collected from the human stomachs. The Light Gradient Boosting Machine algorithm exhibited the best prediction capacity and time efficiency. Haider et al[12] investigated the diagnostic potential of Raman spectroscopy in comparison with RUT and histopathology for *H. pylori* diagnosis. The applicability of Raman spectroscopy as an innovative tool for the molecular detection of *H. pylori* infection was reported. The diagnostic potential of deep convolutional neural networks (DCNNs) for detecting *H. pylori* infection in patients who underwent EGD and CLO tests was assessed, and a total of 13,071 images of various gastric sub-areas were categorized with five pre-trained DCNN architectures employed[13]. The DCNN model appeared promising.

Endoscopy

The diagnostic accuracy of narrow-band imaging (NBI) in predicting *H. pylori* gastritis in dyspepsia was assessed[14]. The presence of abnormal NBI antral mucosal pattern on endoscopy showed excellent diagnostic accuracy in *H. pylori* diagnosis. A comparison of endoscopic images by gastroscopy using WLI and image-enhanced endoscopic (IEE) techniques, such as magnifying narrow-band imaging (NBI), linked color imaging (LCI), and magnifying blue laser imaging (BLI), showed sensitivity was significantly improved with magnifying-BLI than with WLI[15]. A meta-analysis of 34 studies evaluating the diagnostic performance of LCI or WLI in the endoscopic diagnosis of *H. pylori* showed higher sensitivity with LCI in the endoscopic diagnosis of *H. pylori* infection than standard WLI[16].

Rapid Urease Tests (RUT)

The accuracy of combined invasive and noninvasive methods to diagnose *H. pylori* infection in primary care patients was evaluated, with results suggesting RUT and *H. pylori*-IgG ELISA as primary diagnostic screening tools[17]. The combination of gastric nodule and RUT in the diagnosis of *H. pylori* infection in 730 children was investigated and found to be effective in predicting *H. pylori* infection in children[18].

Histology and Deep Learning

Chen et al[19] compared the ability of pathologists to identify *H. pylori* on Hematoxylin and Eosin (H&E) slides using a digital platform with the gold standard of H&E glass slides using routine light microscopy. A significant improvement in the diagnostic accuracy with immunohistochemical slides was observed. Digital whole slide images were found to have some shortcomings in accuracy and precision. The potential of five pre-trained models for binary classification of 204 histopathological images for *H. pylori*-positive and *H. pylori*-negative cases was explored, with ResNet101 emerging as the best[20]. Lin et al[21] developed a two-tier deep-learning-based model for diagnosing *H. pylori* gastritis, and the whole-slide images performed excellently. The clinical applications of Endofaster were evaluated using data from 11 studies, and results suggested that gastric juice analysis may be useful in *H. pylori* diagnosis in patients with chronic active gastritis without evidence of bacteria in histology[22]. Further analysis was done to assess the accuracy of Endofaster in detecting *H. pylori* infection compared to paired histology. Endofaster showed high accuracy for *H. pylori* detection, with moderate agreement with histology[23].

Serology and Pepsinogen (PG) Tests

The diagnostic value of serum *H. pylori* IgG antibody and SAT in the detection of *H. pylori* infections was evaluated, with the variable prevalence of *H. pylori* infection contrasting at a 100% prevalence as observed in the serum immunochromatographic antibody test[24]. The applicability of outer membrane protein-cell receptor reactions in establishing a reliable diagnosis was assessed by Dyankov et al[25]. Surface plasmon resonance (SPR) and double resonance long period grating (DR LPG) biosensors were developed based on the BabA-Le^b binding reaction for diagnosing *H. pylori* infection. SPR showed higher sensitivity than that of the RUT, while the DR LPG biosensor showed superior accuracy and sensitivity. Results from a panel of ten *H. pylori*-specific antibodies in individuals with different *H. pylori* infection status showed that antibody reactivity against cytotoxin-associated gene A (*cag*A), *H. pylori* chaperone (GroEL), and hook-associated protein 2 homologue (FliD) was independently associated with the risk of *H. pylori* exposure, while *H. pylori* adhesin A (HpaA) and γ-glutamyl transpeptidase (gGT) were associated with a current infection[26]. Validation of the *H. pylori* serological and pepsinogen (PG) assays for detecting infection and gastric neoplasm showed the chorus test was more accurate than

the GastroPanel test[27]. Evaluation of the Latex Agglutination Turbidity (LA) assay used for diagnosing *H. pylori* infection and predicting gastric mucosal changes suggested the use of the assay with the PG I/II ratio to avoid missing gastric cancer patients with low LA values[28].

MOLECULAR DETECTION AND ANTIBIOTIC SUSCEPTIBILITY

Invasive Molecular Tests

The use of peptide mass fingerprinting as a molecular tool in the diagnosis of *H. pylori*, in addition to PCR, real-time PCR, and FISH, was recommended in the study by Elbehiry et al[29] while the diagnostic approach of *H. pylori* infection in the era of treatment failure was explored[30]. The latter studies demonstrated how the application of whole genome sequencing (WGS) allowed the detection of more genes than PCR with considerable sequencing depth. The performance of MmaxSure™ *H. pylori* & ClaR Assay (MmaxSure™) in the detection of clarithromycin resistance in *H. pylori* was evaluated with MmaxSure™ showing comparable diagnostic performance with the dual priming oligonucleotide (DPO-PCR) in the detection of the *H. pylori* and A2143G mutation[31]. In a related study, Allplex™ *H. pylori* & ClariR Assay (Allplex™) for detecting clarithromycin resistance in *H. pylori* subjects who underwent EGD was evaluated[32]. Allplex™ and DPO-based multiplex PCR were comparable with direct gene sequencing as the gold standard. The effectiveness of RT-PCR compared to RUT was evaluated and its value was assessed for CLR resistance[33]. RT-PCR assay was found to be more efficient than RUT and could effectively verify CLR resistance. PCR detection of *H. pylori* infection in gastric biopsies allowed its detection and the mutations associated with macrolide resistance[34]. The performance of RIDA®GENE *H. pylori* PCR (r-Biopharm) on the ELITe InGenius System (Elitech) was evaluated for *H. pylori* detection and found to be successful. A rapid visual assay for detecting *H. pylori* and its major virulence genes *(cag*A, *vac*As1, and *vac*Am1) showed more sensitivity than traditional methods[35].

NON-INVASIVE MOLECULAR TESTS

A novel next-generation sequencing (NGS)-based analysis of stool susceptibility testing in comparison with gastric biopsy culture showed that stool NGS-based antimicrobial susceptibility analysis was highly concordant for clarithromycin, levofloxacin, and amoxicillin resistance[36]. A high-throughput multiplex genetic detection assay (HMGA) for *H. pylori* from oral samples was established, with the HMGA confirmed as a reliable assay[37]. A systematic review and meta-analysis for the diagnosis and eradication therapy of *H. pylori* based on gene detection of clarithromycin resistance in stool specimens proved genotypic testing of clarithromycin resistance from stool specimens as an accurate method[38]. Graham[39], in his review, pointed out that the recent availability of susceptibility testing for *H. pylori* infections in the United States resulted in paradigm shifts in the diagnosis. Reflexive stool susceptibility testing, performed when a stool sample tests positive, is rendering current diagnostic and treatment guidelines outdated. These reviews were similar to those reported by Butler et al[40] with few differences. Supplemetary Table 1 shows the sensitivity and specificity of the tests as seen in this review update.

CONCLUSIONS

Diagnosis of *H. pylori* is evolving, and therefore accurate; prompt diagnosis is key to the management of *H. pylori* infections. Diagnostic methods for the detection of *H. pylori* have remarkably improved with accuracy, speed, and efficiency with enhanced UBT, artificial intelligence and machine learning, histology and deep learning, biosensors, microfluidic devices, enhanced endoscopy such as NBI, BLI, and LCI. While molecular methods such as NGS based methods from stool, are likely to be on the increase for the future as these detect both the pathogen and susceptibility testing, negating the problems associated with laborious culture methods.

Conflict of Interest

The authors declare no conflict of interest.

Ethics Statement and Informed Consent

Not applicable due to the type of study.

Informed Consent

Not applicable due to the type of article.

Authors' Contributions

SIS provided most of the literature used in this work, and RAU prepared most of the draft. Both authors reviewed the final version of the article.

AI Statement

The authors did not use AI tools for the preparation of the manuscript.

REFERENCES

1. Malfertheiner P, Camargo C, El-Omar E, Liou J-M, Peek R, Schulz C, Smith SI, Suerbaum S. *Helicobacter pylori*. Nat Rev Dis Primers 2023; 9: 19.
2. Costa LCMC, das Graças Carvalho M, La Guárdia Custódio Pereira AC, Teixeira Neto RG, Andrade Figueiredo LC, Barros-Pinheiro M. Diagnostic Methods for *Helicobacter pylori*. Med Princ Pract 2024; 33: 173-184.
3. Sousa C, Ferreira R, Santosa SB, Azevedo NF, Melo LDR. Advances on diagnosis of *Helicobacter pylori* infections. Crit Rev Microbiol 2023; 49: 671-692.
4. Price TK, Realegeno, Azad Kamran, Shier KL. Validation of the PyloPlus Urea Breath Test System in pediatric patients. Microbiol Spectr 2024; 12: e0318223.
5. Leung WK, Cheung KS, Sham PCO, Tang RSY, Loo CK, Hsu ASI, Cheung TK, Lam LY, Chiu CF, Wu JCY. Consensus recommendations for the screening, diagnosis, and management of *Helicobacter pylori* infection in Hong Kong. Hong Kong Med J 2023; 29: 532-541.
6. Smith SI, Schulz C, Ugiagbe R, Ndip R, Dieye Y, Leja M, Onyekwere C, Ndububa D, Ajayi A, Jolaiya TF, Jaka H, Setshedi M, Gunturu R, Otegbayo JA, Lahbabi-Amrani N, Arigbabu AO, Kayamba V, Nashidengo PA. *Helicobacter pylori* Diagnosis and Treatment in Africa: The First Lagos Consensus Statement of the African Helicobacter and Microbiota Study Group. Dig Dis 2024; 42: 240-256.
7. Omar M, Abu-Salah R, Agbareia R, Sharif Y, Levin R, Lahat A, Sharif K. A comparative systematic review and meta-analysis on the diagnostic accuracy of non-invasive tests for *Helicobacter pylori* detection in elderly patients. Front Med (Lausanne) 2023; 10: 1323113.
8. Shakir SM, Shakir FA, Couturier MR. Updates to the Diagnosis and Clinical Management of *Helicobacter pylori* Infections. Clin Chem 2023; 69: 869-880.
9. Liao EC, Yu CH, Lai JH, Lin CC, Chen CJ, Chang WH, Chien DK. A pilot study of non invasive diagnostic tools to detect *Helicobacter pylori* infection and peptic ulcer disease. Sci Rep 2023; 13: 22800.
10. Shen Y, Chen A, Zhang X, Zhong X, Ma A, Wang J, Wang X, Zheng W, Sun Y, Yue L, Zhang Z, Zhang X, Lin N, Kim JJ, Du Q, Liu J, Hu W. Real-Time Evaluation of *Helicobacter pylori* Infection by Convolution Neural Network During White-Light Endoscopy: A Prospective, Multicenter Study (With Video). Clin Transl Gastroenterol 2023; 14: e00643.
11. Tang JW, Li F, Liu X, Wang JT, Xiong XS, Lu XY, Zhang XY, Si YT, Umar Z, Tay ACY, Marshall BJ, Yang WX, Gu B, Wang L. Detection of *Helicobacter pylori* Infection in Human Gastric Fluid Through Surface-Enhanced Raman Spectroscopy Coupled with Machine Learning Algorithms. Lab Invest 2024; 104: 100310.
12. Haider SI, Akhtar N, Saleem M, Ahmed S, Nadeem S, Amjad M, Haq FU. Diagnosis of *Helicobacter pylori* infection of the gastric biopsy by rapid urease test, histopathology and Raman spectroscopy. Diagn Microbiol Infect Dis 2024; 108: 116129.
13. Kang D, Lee K, Kim J. Diagnostic usefulness of deep learning methods for *Helicobacter pylori* infection using esophagogastroduodenoscopy images. JGH Open 2023; 7: 875-883.
14. Memon HL, Yaseen RT, Khalid MA, Lail G, Shahzad S, UI Haque MM, Abrar G, Khan SA, Laeeq SM, Luck NH. Diagnostic Accuracy of Narrow-Band Imaging in Predicting *Helicobacter pylori* Gastritis in Patients with Dyspepsia. Cureus 2024 16: e54756.
15. Chatrangsun B, Aumpan N, Pornthisam B, Chonprasertsuk S, Siraamolpiwat S, Bhanthumkomol P, Nunanan P, Issarivakulkam N, Mahachai V, Yamaoka Y, Vilaichone. Simultaneous detection of *Helicobacter pylori* infection comparing between white light and image-enhanced endoscopy. BMC Gastroenterol 2024; 24: 46.

16. Lee JG, Yoo IK, Yeniova AO, Lee SP; Research Group for Endoscopic Imaging of Korean Society of Gastrointestinal Endoscopy. The Diagnostic Performance of Linked Color Imaging Compared to White Light Imaging in Endoscopic Diagnosis of *Helicobacter pylori* Infection: A Systematic Review and Meta-Analysis. Gut Liver 2024; 18: 444-456.
17. Duquesne A, Falcón R, Galindo B, Feliciano O, Gutiérrez O, Baldoquín W, Fonseca MC, Llanes R, Sarmiento L. Diagnostic Testing Accuracy for *Helicobacter pylori* Infection among Adult Patients with Dyspepsia in Cuba's Primary Care Setting. Microorganisms 2023; 11: 997.
18. Wu H, Zhou Y, Huang Y. Accuracy of gastric nodule combined with rapid urease test prediction in diagnosing *Helicobacter pylori* infection in children. Eur J Clin Microbiol Infect Dis 2024 43: 481-487.
19. Chen W, Ziebell J, Arole V, Parkinson B, Yu L, Dai H, Frankel WL, Yearsley M, Esnakula A, Sun S, Denise G, Vazzano J, Mishra M, Schoenfield L, Kneile J, Reuss, Schumacher M, Satturwar, Li Z, Parwani A, Lujan G. Comparing Accuracy of *Helicobacter pylori* Identification Using Traditional Hematoxylin and Eosin-Stained Glass Slides with Digital Whole Slide Imaging. Lab Invest 2024; 104: 100262.
20. Ibrahim AU, Dirilenoğlu F, Hacisalihoğlu UP, Ilhan A, Mirzaei O. Classification of *H. pylori* Infection from Histopathological Images Using Deep Learning. J Imaging Inform Med 2024; 37: 1177-1186.
21. Lin YJ, Chen CC, Lee CH, Yeh CH, Jeng YM. Two-tiered deep-learning-based model for histologic diagnosis of *Helicobacter* gastritis. Histopathology 2023; 83: 771-781.
22. Zullo A, Annibale B, Dinis-Ribero M, Fanchellucci G, Esposito G, Hassan C. Gastric juice analysis in clinical practice: why, how, and when. The experience with EndoFaster. Eur J Gastroenterol Hepatol 2024; 36: 264-270.
23. Conti Bellocchi MC, Gasparini E, DI Stefano S, Bobba V, Bernardoni L, Fino V, Crinò SF, Gabbrielli A. The real-time analysis of gastric juice (Endofaster) for detection of *Helicobacter pylori*: a reliable tool in substitution of histology. Minerva Gastroenterol (Torino) 2024; 70: 315-321.
24. Ofariri BAA, Saeed MK, AI-Qubaty M, Abdulkareem AM, AI-Jahrani MA. Diagnostic value of IgG antibody and stool antigen tests for chronic *Helicobacter pylori* infections in Ibb Governorate, Yemen. Sci Rep 2024; 14: 7536.
25. Dyankov G, Eftimov T, Hikoya E, Najdenski H, Kussovski V, Genova-Kalou P, Mankov V, Kisov H, Veselinov P, Ghaffari SS, Kovacheva-Slavova M, Vladimirov B, Malinowski N. SPR and Double Resonance LPG Biosensors for *Helicobacter pylori* BabA Antigen Detection. Sensors (Basel) 2024; 24: 2118.
26. Li ZX, Bronny K, Formichella L, Mejia-Luque, Burrell T, Macke L, Lang U, Vasapolli R, Hysenaj O, Stallforth I, Vieth M, You WC, Zhang Y, Suerbaum S, Schulz C, Pan K-F, Gerhard M. A multiserological line assay to potentially discriminate current from past *Helicobacter pylori* infection. Clin Microbiol Infect 2024; 30: 114-121.
27. Lee SY, Ahn YS, Moon HW. Comparison between the GastroPanel test and the serum pepsinogen assay interpreted with the ABC method-A prospective study. Helicobacter 2024; 29: e13056.
28. Khangai A, Akada J, Saruuliavkhlan B, Gantuya B, Azzaya D, Oyuntsetseg, Davadori D, Uchida T, Matsumoto T, Yamaoka Y. Utilization of an Automated Latex Agglutination Turbidity Assay for Assessing Gastric Mucosal Alteration during *Helicobacter pylori* Infection. Gut Liver 2024; 18: 60-69.
29. Elbehiry A, Marzouk E, Aldubaib M, Abalkhail A, Anagreyyah S, Anajirih N, Almuzaini AM, Rawway M, Alfadhel A, Draz A, Abu-Okail A. *Helicobacter pylori* Infection: Current Status and Future Prospects on Diagnostic, Therapeutic and Control Challenges. Antibiotics 2023; 12: 191.
30. Spagnuolo R, Scarlata GGMS, Paravati MR, Abenavoli L, Luzza F. Change in Diagnosis of *Helicobacter pylori* Infection in the Treatment-Failure Era. Antibiotics (Basel) 2024; 13: 357.
31. Kim I, Shin YR, Kim JS, Kim BW, Maeng LS, Kim JM. Detection of Clarithromycin Resistance in *Helicobacter pylori* Using MmaxSure™ *H. pylori* & ClaR Assay. Dig Dis 2024; 42: 292-298.
32. Kim I, Maeng LS, Kim JS, Kim BW, Cheung DY, Kim JI, Park SH. Correction: Quantitative multiplex real-time polymerase chain reaction assay for the detection of *Helicobacter pylori* and clarithromycin resistance. BMC Microbiol 2023; 23: 188. doi: 10.1186/s12866-023-02937-3. Erratum for: BMC Microbiol 2023; 23: 155.
33. Noh JH, Ahn JY, Choi J, Park YS, Na HK, Lee JH, Jung KW, Kim DH, Choi KD, Song HJ, Lee GH, Jung H-Y, Kim JM. Real-Time Polymerase Chain Reaction for the Detection of *Helicobacter pylori* and Clarithromycin Resistance. Gut Liver 2023; 17: 375-381.
34. Bénéjat L, Ducournau A, Martins CD, Bessède E, Lehours P. RIDA®GENE *Helicobacter pylori* PCR on the ELITe InGenius System. Eur J Clin Microbiol Infect Dis 2023; 42: 593-596.
35. Wang B, Gan Q, Tong Y, Qiao Y, Han M, Zhang R, Han Q, Li C, Bai S, Xu L, Yin Y, Zhang C, Munkhtsetseg B, Zhao X, Meng M, Xi R. A visual diagnostic detection of *Helicobacter pylori* and the gastric carcinoma-related virulence genes (cagA and vacA) by a fluorescent loop-mediated isothermal amplification (LAMP). Talanta 2023; 256: 124260.
36. Bonilla S, Goldsmith J, Mitchell P, Bousvaros A. *Helicobacter pylori* Antimicrobial Resistance Using Next-Generation Sequencing in Stool Samples in a Pediatric Population. J Pediatr Gastroenterol Nutr 2023; 77: 623-627.
37. Chi W, Wang S, Liu T, Jiang W, Ding Li, Miao Y, Yang F, Zhang J, Ji D, Xiao Z, Zhu H, Wu Y, Bao Z, Zhao H, Wang S. A rapid and high-throughput multiplex genetic detection assay for detection, semi-quantification and virulence genotyping of *Helicobacter pylori* in non-invasive oral samples. Front Cell Infect Microbiol 2023; 13: 1267288.
38. Ren X, Shi Y, Suo B, Yao X, Lu H, Li C, Zhang Y, Zhou L, Tian X, Song Z. Individualized diagnosis and eradication therapy for *Helicobacter pylori* infection based on gene detection of clarithromycin resistance in stool specimens: A systematic review and meta-analysis. Helicobacter 2023; 28: e12958.
39. Graham DY. Implications of the paradigm shift in management of *Helicobacter pylori* infections. Therap Adv Gastroenterol 2023; 16: 1-16.
40. Butler TJ, Malloy S, McNara D, Smith SM. Diagnosis of *Helicobacter pylori* infection. Microb Health Dis 2023; 5: e922.