Review

Qinglong Zhang, Shuang Yang, Jianhua Zhou, Zhipeng Li, Lili Wang* and Quanjiang Dong*

# Diagnostic accuracy of stool sample-based PCR in detecting *Helicobacter pylori* infection: a meta-analysis

https://doi.org/10.1515/labmed-2023-0004
Received January 9, 2023; accepted June 19, 2023;
published online July 26, 2023

**Abstract:** The present study was to evaluate the diagnostic accuracy of different types of PCR tests with the aim of determining which one performs best for detecting *Helicobacter pylori* in stool samples. Related articles were searched from PubMed, Embase, Web of Science databases, Scopus, and Scholar Google. The quality of included studies was assessed using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool and RevMan5.4 software. Pooled sensitivity, specificity, DOR, PLR and NLR for the stool PCR test in detecting *H. pylori* infection were performed by Stata 15.0 software. Subgroup meta-analysis was performed by Open Meta-analyst software. Ten studies were selected in this study. Stool PCR test had 92.0 % (83.0, 96.0 %) pooled sensitivity, 96.0 % (84.0, 99.0 %) pooled specificity, 296.0 (51.6, 1,696.9) pooled DOR, 26.1 (5.3, 128.7) pooled PLR and 0.09 (0.04, 0.18) NLR in the diagnosis of *H. pylori* infection, and summary receiver operating characteristic curve (SROC) illustrated an area under the curve (AUC) of 0.98. Subgroup meta-analysis showed rtPCR as having the highest diagnostic accuracy. Our results identify rtPCR as having the highest diagnostic accuracy for the detection of *H. pylori* in stool samples, and the stool PCR test as a reliable diagnostic tool for *H. pylori* infection.

***Corresponding authors: Lili Wang and Quanjiang Dong**, Central Laboratories, Qingdao Municipal Hospital, 266071 Qingdao, P.R. China, E-mail: allyking114@126.com (L. Wang), jiangacer@126.com (Q. Dong)
**Qinglong Zhang,** Central Laboratories, Qingdao Municipal Hospital, Qingdao, P.R. China; and Shandong First Medical University, Jinan, P.R. China, E-mail: along416@126.com
**Shuang Yang,** Fever Clinic, Qingdao Municipal Hospital, Qingdao, P.R. China, E-mail: y.s6622@163.com
**Jianhua Zhou,** Department of Stomatology, Qingdao Municipal Hospital, Qingdao, P.R. China, E-mail: zhoujhqd@126.com
**Zhipeng Li,** Qingdao University, Qingdao, P.R. China, E-mail: qdulzp@163.com

**Keywords:** diagnostic accuracy; *Helicobacter pylori*; meta-analysis; polymerase chain reaction; stool samples

## Introduction

*Helicobacter pylori* (*H. pylori*) is a Gram-negative bacterium that is associated with various disorders, such as chronic gastritis, MALT lymphoma and gastric cancer [1]. Considering that more than half of the world's population is infected with *H. pylori*, the accurate detection of this bacteria is essential for the treatment of patients and the confirmation of successful eradication post-treatment [2]. The diagnostic tests for *H. pylori* infection can be classified as either invasive or non-invasive and are often used in combination to achieve a better diagnostic result. Invasive methods are based on endoscopy and the testing of biopsied tissue, and include the rapid urease test (RUT), bacterial culture, and histologic examination. Non-invasive methods include the detection of bacterial urease activity via urea breath test (UBT), antibody-based testing and stool antigen detection (SAT) [3]. The gold standard for the diagnosis of *H. pylori* infection is bacterial culture, however, it is an invasive procedure requiring endoscopic gastric biopsy samples and an incubation period of at least 4–7 days [4]. Since these invasive methods cause discomfort to patients, non-invasive methods are more commonly used in clinical practice.

Polymerase chain reaction (PCR) is a reliable detection method that is widely used in biological and medical research. In recent years, the use of PCR for the detection of *H. pylori* infection has attracted the attention of many researchers. Different PCR-based methodologies have been developed and are widely used, including conventional PCR, nested PCR and real-time PCR (rtPCR) [5]. For rtPCR, the probes and primers are highly sequence specific and the degree of amplification during the PCR reaction can be quantified by conducting a melting curve. Additionally, this method is quick and reliable [6]. Nested PCR involves the use of two primer sets directed against the same target, and two

successive PCR reactions. In the case of *H. pylori* detection, PCR-based tests have been successfully used with gastric tissue samples, as well as direct detection from stool samples [7]. Previous study of meta-analysis [8] demonstrated that the sensitivity and specificity of stool PCR tests are relatively similar in comparison to other diagnostic methods for detecting *H. pylori* infection. While the study not use bacterial culture or the composite reference method (CRM) to assessing the heterogeneity among selected literature, possibly leading to less reliable conclusions. In recent years, a growing number of PCR tests based on rtPCR or nested PCR have shown high diagnostic performance and have been used to detect *H. pylori* infection. UBT is currently a reliable non-invasive test for the diagnosis of *H. pylori* infection. However, UBT has many requirements for patients including fasting, compliance and adequate functions of important organs [3]. In contrast, fecal samples for stool PCR test are easier to collect. Importantly, stool PCR can simultaneously detect point mutations resulting in antibiotic resistance, which improve the success rate of initial eradication therapies in patients with *H. pylori* infection [7]. So far, stool PCR based diagnostic methods have become more commonly available. Despite the recent developments and many advantages of PCR, studies to date have not yet revealed which PCR method is best for the detection of *H. pylori* in stool samples.

In this study, we performed a more comprehensive meta-analysis than previous studies in that we referred to the Preferred Reporting Items for a Systematic Review and Meta-Analysis of Diagnostic Test Accuracy Studies (PRISMA-DTA) guidelines [9] and summarised the literature that used bacterial culture or CRM as the standard. In this way, we were able to determine the type of PCR with the best diagnostic performance in detecting the presence of *H. pylori* in stool samples. Additionally, we also evaluated the diagnostic value of the stool PCR test.

# Methods

The protocol of our study was based on the PRISMA-DTA guidelines [9].

### Literature search strategy

To identify relevant studies, we executed a comprehensive search of the following three electronic databases: PubMed (www.PubMed.gov), Embase (www.Embase.com), Web of Science (www.webofscience.com), Scopus (www.scopus.com) and Scholar Google (www.scholar.google.com) from January 1, 2000 to June 1, 2022. The following keyword search terms were used: "(stool OR faecal OR faeces)" AND "(*Hp* OR *H. pylori* OR *H. pylori*)" AND "(PCR OR Polymerase chain reaction)". In order to avoid missing any published studies, our search was not limited by date or type of publication.

### Study selection

Two reviewers (Zhang QL and Yang S) independently assessed all identified publications to determine their eligibility for inclusion in the study. Those that satisfied the following criteria were included in the sample: (1) patients infected with *H. pylori*; (2) stool samples were collected immediately before therapy was administered; (3) provided sufficient data for $2 \times 2$ table construction for true positive (TP), false positive (FP), false negative (FN), and true negative (TN) determination; (4) culture or CRM to detect the presence of *H. pylori* was utilized; (5) detection of *H. pylori* target genes was performed, including 23S rRNA, 16S rRNA, urease gene, *glmM* and *hsp60*; (6) the full text of the included literature is in English.

Exclusion criteria were as follows: (1) animal experiments; (2) duplicate publications; (3) reviews, case reports and letters; (4) study population consisted of only pediatric patients; (5) complete data for TP, FP, FN, and TN were not available.

### Data extraction

Two independent reviewers (Zhang QL and Dong QJ) carefully extracted the following information from each of the selected studies: name of the first author, published year, sample size, standard of *H. pylori* infection diagnosis, target genes, PCR type, region of the study population, TP, FP, FN and TN numbers. The $2 \times 2$ tables were then used to calculate the sensitivities and specificities of the target assays. A third independent reviewer (Wang LL) helped to resolve any disagreements between the two reviewers during the data extraction process.

### Methodological quality

A quality assessment was carried out to analyse the presence and impact of bias among the included studies. To do this, two authors (Zhang QL and Li ZP) independently used the QUADAS-2 tool [10] to evaluate four key domains: patient selection, index test, reference standard, flow and timing. In case of uncertainty, discussion was conducted until a consensus was met. For each domain, the risk of bias was analysed using several signalling questions. In addition to the risk of bias, the tool also evaluated the applicability of each included study to the research question. According to QUADAS-2, the risk of bias and applicability was rated high, low, or unclear, and the analysis was conducted using RevMan5.4 software (Cochrane Collaboration, Oxford, UK). Deeks' funnel plot asymmetry test was used to estimate publication bias [11] and was performed by Stata15.0 software (College Station, TX, USA).

### Statistical analysis

The meta-analysis was carried out using bivariate random effects model [12] and Stata15.0 software. To estimate the capability of stool-based PCR in detecting *H. pylori* infection, we calculated: pooled sensitivity, specificity, diagnostic odds ratio (DOR), area under the curve (AUC), positive likelihood ratio (PLR) and negative likelihood ratio (NLR).

To assess the heterogeneity among studies, $I^2$ values and Q-test were calculated, with a $I^2$ value greater than 50 % or p-value <0.1 for Q-test indicating substantial heterogeneity [13]. Furthermore, subgroup meta-analysis was performed to find the reason for heterogeneity and estimate the accuracy of different type of PCR in detection of *H. pylori* in stool samples. Subgroup meta-analysis was performed by Open Meta-analyst software (Brown University, USA). Subgroup meta-analysis was used to analyse whether heterogeneity is caused by non-threshold effects, and p-value <0.05 was considered statistically significant. Sensitivity, specificity, DOR and $I^2$ value were obtained using the diagnostic random effects model and the DerSimonian–Laird method. Finally, heterogeneity between subgroups was assessed with a Q-test.

# Results

## Study inclusion

Electronic search identified a total of 2,739 records from the following databases: PubMed (512 records), Embase (588 records), Web of Science (927 records), Scopus (363 records) and Scholar Google (349 records) (Figure 1). After removal of duplicate publications and detailed analysis of the selected articles, we identified 56 that were related to the diagnosis of *H. pylori* infection via stool PCR test. Of these, 46 studies were excluded from further analysis due to lack of data to be extracted (23), as the diagnosis of the infection did not meet the required criteria (11) or the study population consisted of only pediatric patients (12). Finally, 10 studies [14–23] in total were included in the meta-analysis.

## Characteristics of the included studies

A total of 2,136 participants were included in the study of *H. pylori* detection by stool PCR. These studies were conducted in nine countries, with a sample size ranging from 50 to 1,086. The majority of studies (80.0 %) used bacterial culture as the standard for *H. pylori* infection. One study used bacterial culture only as the standard, and the remaining nine studies used CRM for the infection. Six studies used bacterial culture plus other tests as the standard. Different amplification methods were used for detection, with nested PCR used in four studies and rtPCR used in five. The target gene to be detected was the 23S rRNA gene in four studies. Other target genes included *ureA*, *ureB*, *ureC* and *hsp60*. Critical data were successfully extracted from all selected studies, including TP, FP, FN, and TN (Table 1).

## Methodological quality of included studies

The majority of included studies were of high quality in terms of patient selection, index test and standard (Figure 2). For the “Patient selection” domain, experiments by Noguchi N et al. [20] showed an increased risk of bias because they did not avoid case-control design. For the “Flow and timing” domain, data from only a portion of patients were included for analyses in studies by Beckman E et al. [18] and Schabereiter-Gurtner C et al. [21]. The reason for this was a lack of acceptable standards for some patients.

Deeks’ funnel plot asymmetry test was used to assess publication bias. As shown in Figure 3, no significant publication bias was evident (p=0.18).

## Overall performance

For the detection of *H. pylori* infection, stool PCR test had a pooled sensitivity of 92.0 % and a pooled specificity of 96.0 %, with 95 % confidence interval (CI) (83.0–96.0) and (84.0–99.0), respectively (Figure 4). The DOR of the stool PCR test was as high as 296.0 (95 % CI: 51.6–1,696.9), indicating a high diagnostic accuracy (Figure 5). There was significant heterogeneity across the studies in both sensitivity and specificity (Figure 4), with $I^2$ greater than 50 % in both cases. A sensitivity below 92.0 % was found in 40.0 % of data points (4/10), and similarly, 40.0 % of data points (4/10) had specificity below 96.0 %. The pooled PLR of stool PCR test was 26.1 (95 % CI: 5.3–128.7), and the pooled NLR was 0.09 (95 % CI: 0.04–0.18) (Figures 6 and 7). The AUC of the stool PCR test was 0.98, indicating a good discriminatory ability of stool PCR testing in detecting *H. pylori* infection (Figure 6). Putting these results together indicates that stool PCR is a test with high diagnostic accuracy. As significant heterogeneity existed in every analysis, we carried out subgroup analysis, described below.

## Subgroup meta-analysis

In order to determine the reason for heterogeneity and to identify the best type of PCR for the detection of *H. pylori* in stool samples, a subgroup meta-analysis was performed based on the different PCR type and target gene.

The results of this subgroup meta-analysis are shown in Tables 2 and 3. Firstly, based on the different PCR types, the included studies were divided into three subgroups:

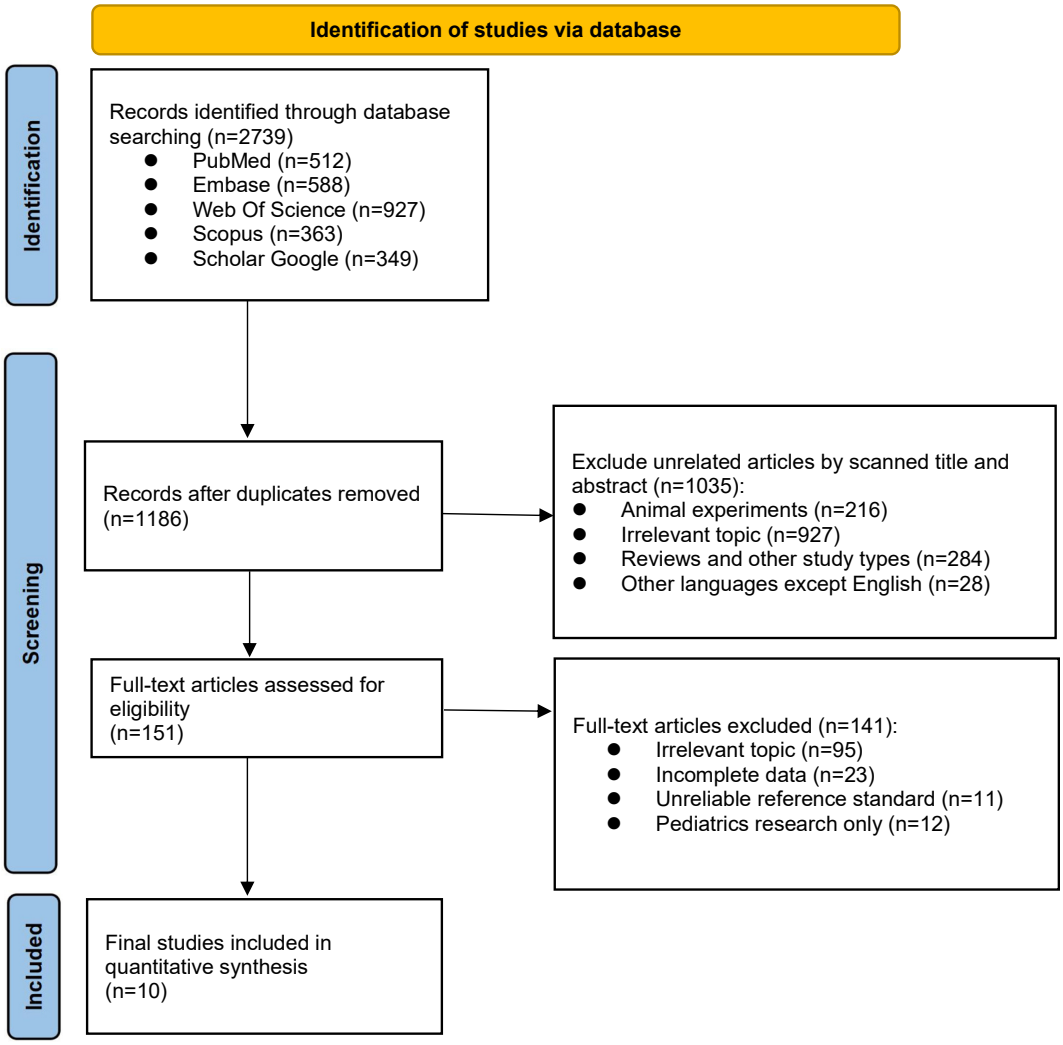


**Figure 1:** Flow-chart for articles identified and analyzed in this meta-analysis.

rtPCR subgroup, nested PCR subgroup and conventional PCR subgroup. The result showed that diagnostic accuracy was increased for stool rtPCR test in terms of DOR, sensitivity and specificity in relation to the nested PCR subgroup (Tables 2 and 3). In sensitivity, Q value of rtPCR subgroup was 7.0 ($p>0.05$), and $I^2$=42.8 %, indicating low heterogeneity. In specificity, subgroup meta-analysis reported a Q value of 5.7 ($p=0.13$) and $I^2$=46.9 % for the nested PCR subgroup indicating low heterogeneity. Finally, based on the different target genes amplified, we divided the included studies into the following subgroups: 23S rRNA, urease gene and *hsp60*. From this, we found the best diagnostic accuracy in DOR, sensitivity and specificity to be when the 23S rRNA was the selected target gene (Tables 2 and 3). The $I^2$ value in sensitivity was low for the 23S rRNA subgroup ($I^2$=0 %).

## Discussion

Making an early and accurate diagnosis is critical for improving the treatment of patients with *H. pylori* infection. In this study, we evaluated the performance of stool PCR test in the detection of *H. pylori*. Our results demonstrated it is an accurate test for the diagnosis of *H. pylori* infection. With respect to invasive tests for the diagnosis of *H. pylori* infection, histology and culture methods have good performance in detecting the bacterium [24, 25]. Recently, a method that directly detects the bacterium under the endoscopy examinations has been reported [26]. Combined color imaging and blue laser imaging in endoscopy examinations have been well evaluated for the diagnosis of *H. pylori* gastritis [27, 28]. *H. pylori* infection can be diagnosed directly

**Table 1:** Baseline characteristics of studies included in the meta-analysis.

| Author | Year | Country | Detection standard | PCR type | Multiplex PCR test | Gene | Sample size | Hp+ | Hp− | Positive rate, % | TP | FP | FN | TN | SEN, % | SPE, % | PCR reagent source | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | | | | CE labelled | Cartridge system | Laboratories themselves |
| Kovacheva-Slavova et al. [14] | 2021 | Bulgaria | UBT, HpSA | rtPCR | Y | *UreB* | 50 | 24 | 26 | 48 % | 24 | 0 | 4 | 22 | 86 % | 100 % | N | Y | N |
| Pichon et al. [15] | 2020 | France | Culture, PCR | rtPCR | Y | 23SrRNA | 1,086 | 160 | 926 | 15 % | 154 | 13 | 6 | 913 | 96 % | 99 % | Y | Y | N |
| Pavoni et al. [16] | 2019 | Italy | UBT, RUT, histology, culture | rtPCR | Y | 23SrRNA | 93 | 69 | 24 | 74 % | 64 | 0 | 5 | 24 | 93 % | 100 % | NA | NA | NA |
| Taborda et al. [17] | 2018 | Chile | Histology, RUT | Nested PCR | N | *UreC* | 102 | 71 | 31 | 69.61 % | 71 | 5 | 0 | 26 | 100 % | 84 % | N | N | Y |
| Beckman et al. [18] | 2017 | Italy | Histology, RUT, culture | rtPCR | Y | 23SrRNA | 290 | 242 | 48 | 83.45 % | 227 | 11 | 15 | 37 | 94 % | 77 % | N | N | Y |
| Mishra et al. [19] | 2008 | India | PCR RUT | Nested PCR | N | *hsp60* | 52 | 40 | 12 | 76.92 % | 29 | 0 | 11 | 12 | 73 % | 100 % | N | N | Y |
| Noguchi et al. [20] | 2007 | Japan | Culture | Nested PCR | N | 23SrRNA | 114 | 108 | 6 | 94.74 % | 100 | 4 | 8 | 2 | 93 % | 33 % | N | N | Y |
| Schabereiter-Gurtner et al. [21] | 2004 | Austria | Culture, histology, RUT | rtPCR | N | *UreA* | 90 | 45 | 45 | 50 % | 44 | 1 | 1 | 44 | 98 % | 98 % | N | N | Y |
| Wiśniewska et al. [22] | 2002 | Poland | Culture, histology | SemiNested PCR | N | *UreA* | 155 | 68 | 87 | 43.87 % | 41 | 14 | 27 | 73 | 60 % | 84 % | N | N | Y |
| Monteiro et al. [23] | 2001 | France | Culture, histology, urease test | PCR | N | *UreA* | 104 | 47 | 57 | 45.19 % | 38 | 0 | 9 | 57 | 81 % | 100 % | N | N | Y |

rtPCR, real-time PCR; UBT, urea breath test; HpSA, *H. pylori* stool antigen test; SEN, sensitivity; SPE, specificity; RUT, rapid urease test; TP, true positive; FP, false positive; FN, false negative; TN, true negative; Y, yes; N, no; NA, not applicable. In the study by Schabereiter-Gurtner et al. [21], two patients did not fulfill the criteria for a definite characterization of their *H. pylori* status, so they were not considered for statistical evaluations.

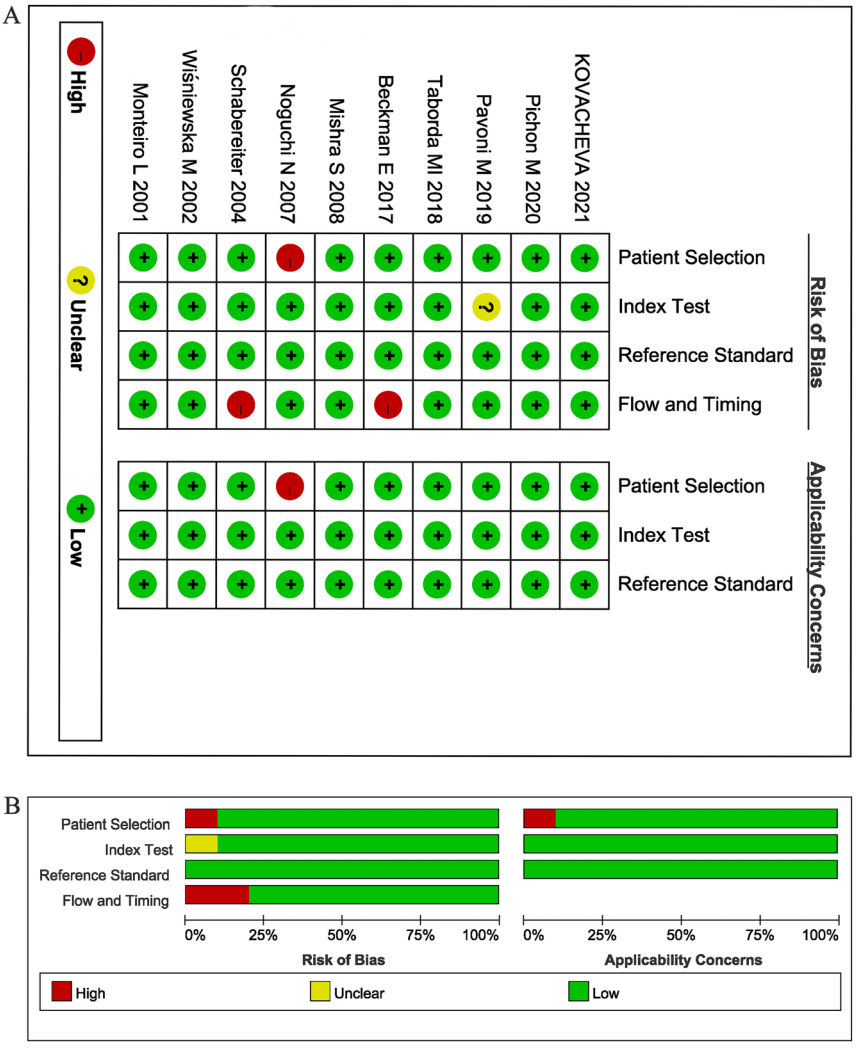


**Figure 2:** Quality assessment for each included study. (A) Risk-of-bias and applicability concerns summary (B) Risk-of-bias and applicability concerns graph. Symbols: (+), low risk of bias (?), unclear risk of bias (–), high risk of bias.

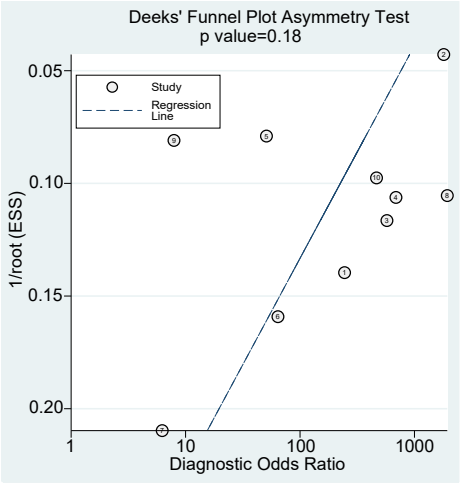


**Figure 3:** Estimation of the publication bias by Deeks' funnel plots for involved trials.

by observing the fine structure of the gastric mucosa. Furthermore, the accuracy of *H. pylori* detection can be improved by taking biopsies at characteristic sites and validated with further histological tests or RUT. With respect to non-invasive tests RUT and monoclonal SAT tests have been recommended as non-invasive method for detection of *H. pylori* [29, 30]. Considering the increasing prevalence of antibiotic resistant strains of *H. pylori*, guideline for management of *H. pylori* infection suggests it is reasonable to perform antimicrobial resistance tests even before the prescribing of the first-line therapies [29]. However, UBT and SAT tests are not capable of detecting antibiotic resistance of *H. pylori*. PCR test, as well as next generation sequencing and bacteria culture methods can simultaneously detect *H. pylori* and bacteria susceptibility to antibiotics [31]. Antimicrobial sensitive tests on bacteria require well-trained personnel, and adequate facilities for sample transport and culture [32].

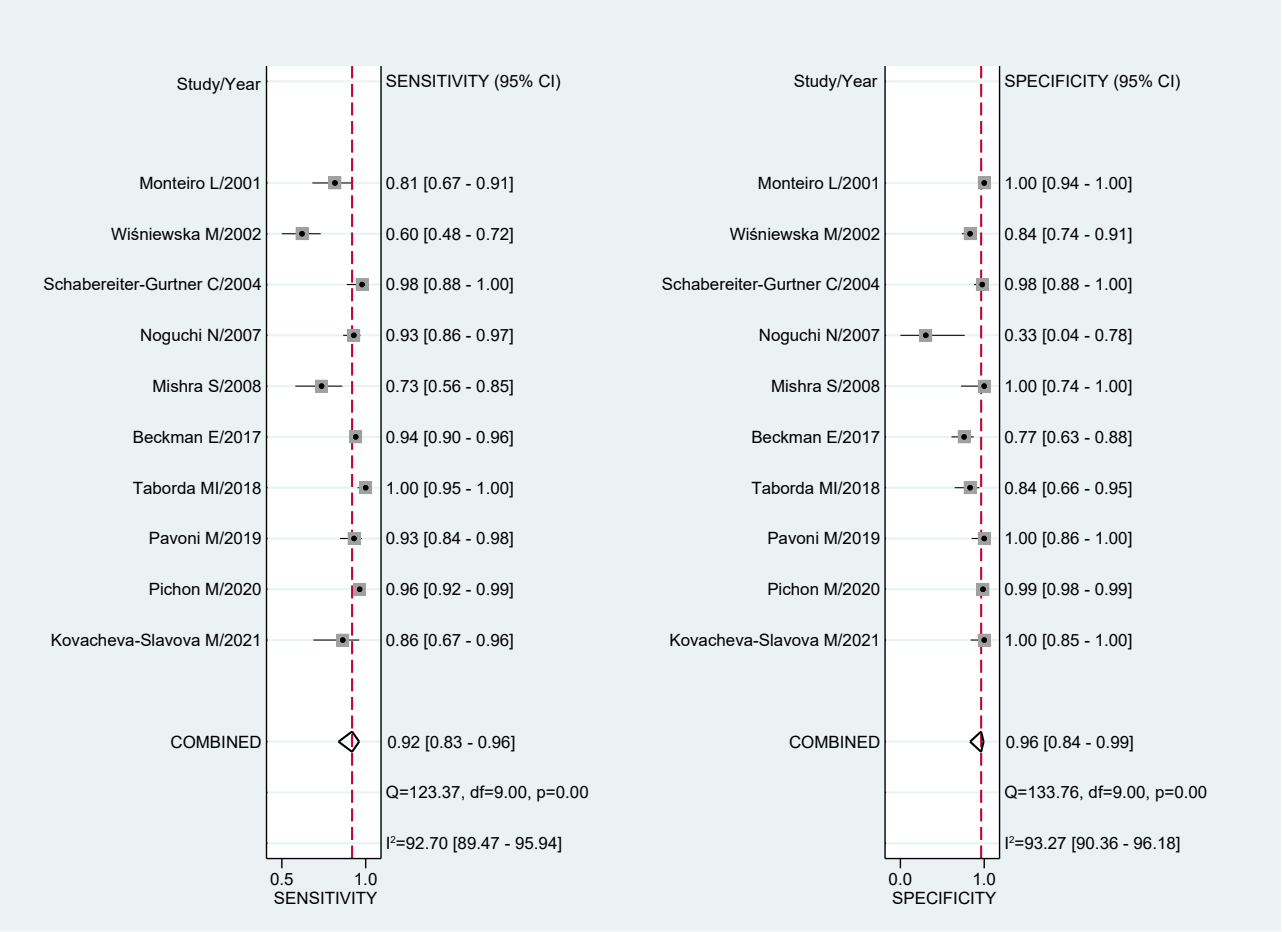


**Figure 4:** Forest plots showing pooled sensitivity and specificity of stool PCR test for diagnosis of *H. pylori* infection. The squares and horizontal lines represent the value of sensitivity or specificity and 95 % CI, respectively; the diamond represents the combined value of sensitivity or specificity and corresponding 95 % CI; Q, value of Q-test for heterogeneity test; df, degrees of freedom; p, p-value for Q-test; $I^2$, I square value for heterogeneity test.

In clinical practice, antimicrobial susceptibility tests are time consuming, expensive and laborious. Therefore they not routinely performed in medical institutions [33]. Next-generation sequencing of bacteria DNA in biopsy samples is a molecular test to detect the bacterium and mutations responsible for resistance to antibiotics [34]. It permits more precise prediction of antibiotic resistance phenotypes, including those with many contributing mutations, such as metronidazole or amoxicillin resistance [35]. However, its high cost, complex procedures and laborious work restrict its currently wide use in the clinical practice. Stool PCR test is a promising non-invasive approach for diagnosis of *H. pylori* infection, considering its high accuracy, convenience for sample collection, less requirement for patients (i.e., fasting, health conditions, compliance), low cost [36].

Although non-invasive diagnostic methods for *H. pylori* infection already exist, there remains room for improvement and a need for more reliable methods. PCR testing of stool samples is a promising approach to replace more invasive testing, as patients are only required to provide a small sample of fresh stool. By eliminating the need for invasive testing, such as gastroscopy, patients are spared the discomfort and cost of the invasive treatment. In this study, our main objective was to determine which type of stool PCR testing performs best in the diagnosis of *H. pylori* infection, and to evaluate the diagnostic value of stool PCR testing.

After demonstrating that no risk of publication bias existed in the included studies, we determined the pooled sensitivity and specificity of stool PCR tests to be 92.0 % (95 % CI: 83.0–96.0) and 96.0 % (95 % CI: 84.0–99.0). The DOR gives a measure of accuracy, combining into a single indicator the strengths of both sensitivity and specificity, with higher values indicating better discrimination in the test performance. In this meta-analysis, the mean DOR was 296.0 (95 % CI: 51.6–1,696.9), indicating a high level of overall accuracy. Likelihood ratios are indices that take into account the interaction between sensitivity and specificity, and a PLR>10 and NLR<0.1 are considered to be convincing evidence to

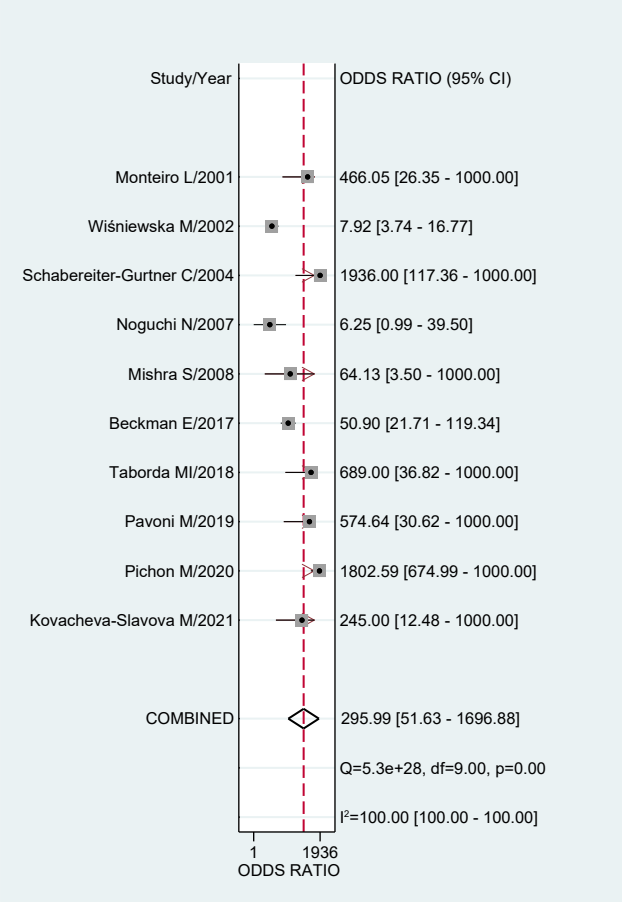


**Figure 5:** Forest plot showing pooled DOR of stool PCR test for diagnosis of *H. pylori* infection. The squares and horizontal lines represent the value of DOR and 95 % CI, respectively; The diamond represents the combined value of DOR and corresponding 95 % CI; Q, value of Q-test for heterogeneity test; df, degrees of freedom; p, p-value for Q-test; $I^2$, I square value for heterogeneity test.

confirm or exclude a diagnosis, respectively. In this study, the pooled PLR of stool PCR tests was 26.1, suggesting that the likelihood of a positive stool PCR test result was about 20-fold higher in patients with *H. pylori* infection than in patients without. The pooled NLR of stool PCR tests was 0.09, indicating that in the case of a negative result, the likelihood of that patient having *H. pylori* infection was low. We used an SROC curve to summarise the overall diagnostic performance of the included studies and found an AUC of 0.98, indicating good diagnostic performance. Putting these results together, we can see that PCR of stool samples is an accurate and valid testing approach for the detection of *H. pylori*. After confirming the important role that PCR can play in the diagnosis of *H. pylori* infection, we went on to assess the type of PCR with the best diagnostic performance for *H. pylori* in stool samples. Our results led us to the following conclusion: rtPCR has the highest diagnostic accuracy among PCR methods for detecting the infection status of *H. pylori* in stool samples. Compared with nested PCR, the diagnostic accuracy was higher with the stool rtPCR test in term of DOR, sensitivity and specificity. As for conventional PCR subgroup, the results were not convincing because only one study was included, so it was not used for comparison. This result is consistent with the suggestion that rtPCR has the best diagnostic performance in the detection of *H. pylori* infection.

Before applying an accurate test to clinical diagnosis, a comparison with current standards is required to evaluate its diagnostic value. This means that the selection of an appropriate gold standard is a prerequisite for the correct evaluation of the diagnostic accuracy of the test in question. In comparison to previous studies, we chose to use stricter reference standards for the diagnosis of *H. pylori* infection, and to exclude those carried out on children. In choosing the standard for inclusion in our meta-analysis, we decided on bacterial culture. In the case of included studies which did not use bacteria culture, we chose CRM as the standard instead. This was done to reduce bias and improve the reliability of the final result.

Heterogeneity was present among the included studies and in order to determine the source, we performed subgroup meta-analysis. We found that the nested PCR subgroup and urease gene subgroup may be the cause of heterogeneity in sensitivity, and rtPCR subgroup may be the cause of heterogeneity in specificity. This means that the presence of heterogeneity could be explained by the PCR type and target gene.

Despite the heterogeneity, we still confidently conclude that rtPCR is the best type of PCR for the diagnosis of *H. pylori* infection in stool samples. As well as being quick and reliable, rtPCR has an additional advantage over nested PCR, with previous studies showing that stool rtPCR with 23S rRNA as the target gene can detect clarithromycin resistance with high diagnostic accuracy [37, 38]. This is of importance, as clarithromycin is the first-line treatment for *H. pylori* infection [39], and resistance to this drug is gradually increasing worldwide [40]. Genetic analysis of resistant strains identified mutations in the 23S rRNA as the predominant cause of resistance [41], with point mutations A2142G, A2143G and A2142C being responsible for over 90 % of cases. These point mutations, and thus clarithromycin susceptibility, can be detected by rtPCR at the same time as testing for *H. pylori*, making its use in the clinical setting important for both detecting *H. pylori* infection quickly and accurately, as well as reducing the abuse of antibiotics.

Although the strength of this meta-analysis is founded on our comprehensive analysis of high-quality studies, it is important to acknowledge the limitations. First, while

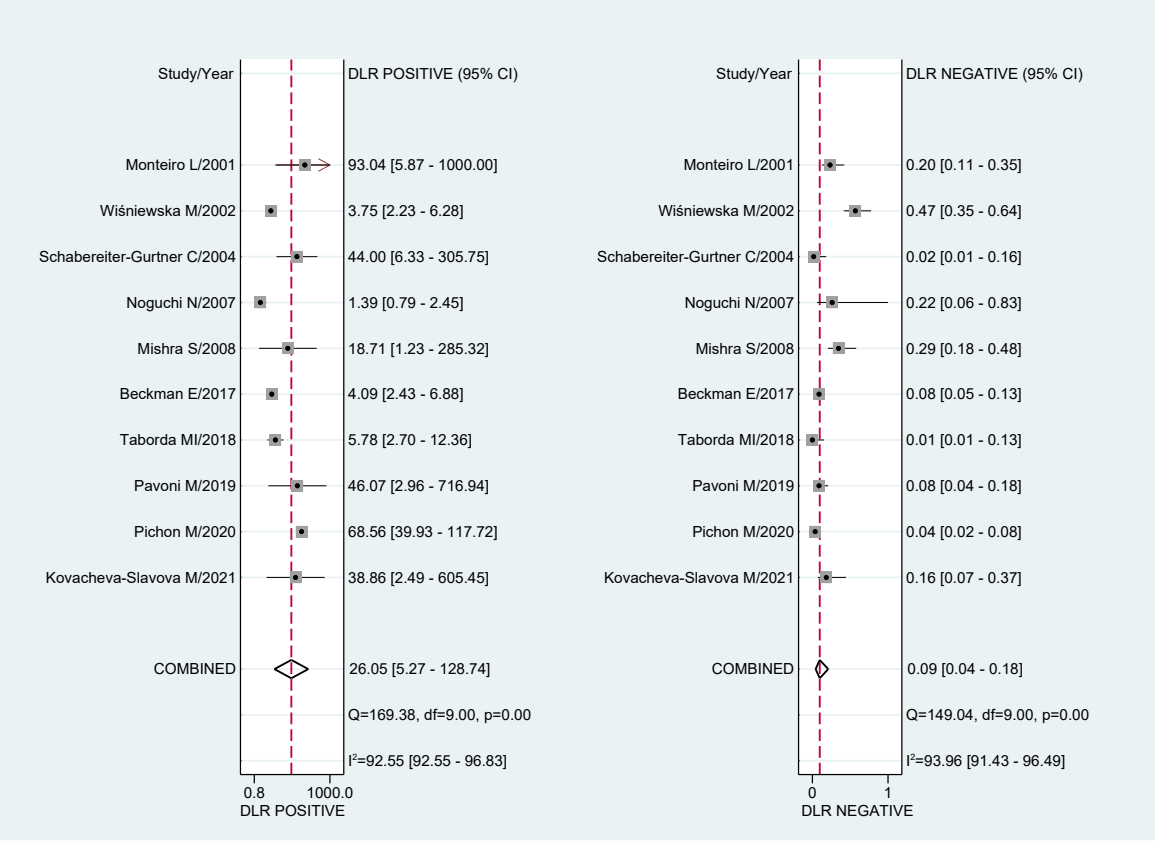


**Figure 6:** The SROC of stool PCR test based on sensitivity and specificity. AUC, area under the curve; SROC, summary receiver operating characteristic.

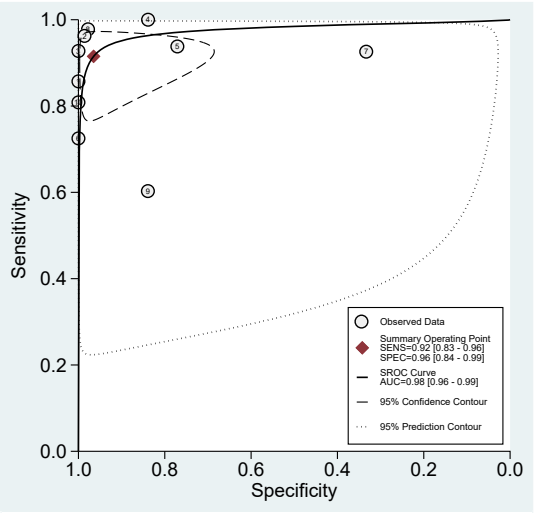


**Figure 7:** Forest plot showing PLR and NLR of stool PCR test for diagnosis of *H. pylori* infection. DLR, diagnostic likelihood ratio. The squares and horizontal lines represent the value of PLR or NLR and 95 % CI, respectively. The diamond represents the combined value of PLR or NLR and corresponding 95 % CI; Q, value of Q-test for heterogeneity test; df, degrees of freedom; p, p-value for Q-test; $I^2$, I square value for heterogeneity test.

**Table 2:** Subgroup meta-analysis based on DOR.

| | Subgroups | Studies | DOR (p) | Q (df) | $I^2$, % (p) |
|---|---|---|---|---|---|
| **PCR type** | Subgroup rtPCR | 5 | 446.9 (<0.001) | 31.2 (4) | 87.2 (<0.001) |
| | Subgroup nested PCR | 4 | 25.2 (<0.001) | 10.2 (3) | 70.5 (0.017) |
| | Subgroup conventional PCR | 1 | 466.1 (NA) | NA | NA |
| **Target gene** | Subgroup 23S rRNA | 4 | 237.6 (<0.001) | 30.5 (3) | 90.1 (<0.001) |
| | Subgroup urease gene | 5 | 221.3 (<0.001) | 29.0 (4) | 86.2 (<0.001) |
| | Subgroup *hsp60* | 1 | 64.1 (NA) | NA | NA |

DOR, diagnosis odd ratio; NA, not applicable; df, degrees of freedom.

we collected the literature as completely as possible, our strict inclusion criteria resulted in the inclusion of only 10 publications. This means the study may have lacked sufficient statistical power to draw a definite conclusion, and more clinical diagnostic studies are needed to reach a final conclusion. Second, some well-designed studies had to be excluded because they were published in a non-English

**Table 3:** Subgroup meta-analysis based on sensitivity and specificity.

| | Subgroups | Studies | SEN, % (p) | Q (df) | $I^2$, % (p) | SPE, % (p) | Q (df) | $I^2$, % (p) |
|---|---|---|---|---|---|---|---|---|
| **PCR type** | Subgroup rtPCR | 5 | 93.5 (<0.001) | 7.0 (4) | 42.8 (0.136) | 96.4 (<0.001) | 48.2 (4) | 91.7 (<0.001) |
| | Subgroup nested PCR | 4 | 85.1 (0.007) | 30.3 (3) | 90.1 (<0.001) | 79.0 (0.008) | 5.65 (3) | 46.9 (0.13) |
| | Subgroup conventional PCR | 1 | 80.2 (NA) | NA | NA | 99.1 (NA) | NA | NA |
| **Target gene** | Subgroup 23S rRNA | 4 | 93.9 (<0.001) | 2.2 (3) | 0 (0.53) | 93.7 (0.01) | 49.2 (3) | 93.9 (<0.001) |
| | Subgroup urease gene | 5 | 87.2 (<0.001) | 23.9 (4) | 83.3 (<0.001) | 92.3 (<0.001) | 10.5 (4) | 61.8 (0.03) |
| | Subgroup *hsp60* | 1 | 72 (NA) | NA | NA | 96.2 (NA) | NA | NA |

SEN, sensitivity; SPE, specificity; NA, not applicable; df, degrees of freedom.

language, resulting in language bias. The inclusion of studies published in languages other than English, as well as those with null results, may have yielded more representative conclusions. Finally, due to the unavailability of data, we were unable to analyse the patient demographics in detail. In the future, we believe it would be of interest to investigate the relationship between *H. pylori* infection and clinical-pathologic characteristics such as patient gender and age.

## Conclusions

Based on the current available evidence, our results demonstrated that rtPCR has the highest diagnostic accuracy in detecting the infection status of *H. pylori* in stool samples, and that stool PCR testing is an accurate and consistent approach to detecting *H. pylori* infection. Therefore, stool rtPCR should be considered feasible in clinical practice as a promising method for quickly, easily, and non-invasively detecting the infection status of *H. pylori.*

**Research funding:** None declared.
**Author contributions:** All authors have accepted responsibility for the entire content of this manuscript and approved its submission.
**Competing interests:** Authors state no conflict of interest.
**Informed consent:** Not applicable.
**Ethical approval:** Not applicable.

## References

1. Fischbach W, Malfertheiner P. Helicobacter pylori infection. Dtsch Arzteblatt Int 2018;115:429–36.
2. Zamani M, Ebrahimtabar F, Zamani V, Miller WH, Alizadeh-Navaei R, Shokri-Shirvani J, et al. Systematic review with meta-analysis: the worldwide prevalence of Helicobacter pylori infection. Aliment Pharmacol Ther 2018;47:868–76.
3. El-Shabrawi M, El-Aziz NA, El-Adly TZ, Hassanin F, Eskander A, Abou-Zekri M, et al. Stool antigen detection vs. (13)C-urea breath test for non-invasive diagnosis of pediatric Helicobacter pylori infection in a limited resource setting. Arch Med Sci 2018;14:69–73.
4. Grove DI, Koutsouridis G, Cummins AG. Comparison of culture, histopathology and urease testing for the diagnosis of Helicobacter pylori gastritis and susceptibility to amoxycillin, clarithromycin, metronidazole and tetracycline. Pathology 1998;30:183–7.
5. Lei S, Gu X, Xue W, Rong Z, Wang Z, Chen S, et al. A 4-plex droplet digital PCR method for simultaneous quantification and differentiation of pathogenic and non-pathogenic Vibrio parahaemolyticus based on single intact cells. Front Microbiol 2020;11:1727.
6. Sulo P, Šipková B. DNA diagnostics for reliable and universal identification of Helicobacter pylori. World J Gastroenterol 2021;27: 7100–12.
7. Qiu E, Li Z, Han S. Methods for detection of Helicobacter pylori from stool sample: current options and developments. Braz J Microbiol 2021; 52:2057–62.
8. Khadangi F, Yassi M, Kerachian MA. Review: diagnostic accuracy of PCR-based detection tests for Helicobacter Pylori in stool samples. Helicobacter 2017;22:e12444.
9. McInnes MDF, Moher D, Thombs BD, McGrath TA, Bossuyt PM, Clifford T, et al. Preferred reporting items for a systematic review and meta-analysis of diagnostic test accuracy studies: the PRISMA-DTA statement. JAMA 2018;319:388–96.
10. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med 2011;155:529–36.
11. Deeks JJ, Macaskill P, Irwig L. The performance of tests of publication bias and other sample size effects in systematic reviews of diagnostic test accuracy was assessed. J Clin Epidemiol 2005;58:882–93.
12. Gardiner JC, Luo Z, Roman LA. Fixed effects, random effects and GEE: what are the differences? Stat Med 2009;28:221–39.
13. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003;327:557–60.
14. Kovacheva-Slavova M, Valkov H, Angelov T, Tropcheva R, Vladimirov B. Screening for Helicobacter pylori infection and clarithromycin resistance using real-time polymerase chain reaction. Eur Rev Med Pharmacol Sci 2021;25:5042–6.
15. Pichon M, Pichard B, Barrioz T, Plouzeau C, Croquet V, Fotsing G, et al. Diagnostic accuracy of a noninvasive test for detection of Helicobacter pylori and resistance to clarithromycin in stool by the amplidiag H. pylori+ClariR real-time PCR assay. J Clin Microbiol 2020; 58:e01787-19.
16. Pavoni M, Turello G, Saracino IM, Fiorini G, Saccomanno L, Lazzarotto T, et al. PCR test for Helicobacter pylori detection and clarithromycin resistance prediction on fecal and bioptic samples: comparison of accuracies. Dig Liver Dis 2019;51:106–7.
17. Taborda MI, Aquea G, Nilo Y, Salvatierra K, López N, López S, et al. Non-invasive diagnostic of Helicobacter pylori in stools by nested-qPCR. Pol J Microbiol 2018;67:11–8.

18. Beckman E, Saracino I, Fiorini G, Clark C, Slepnev V, Patel D, et al. A novel stool PCR test for Helicobacter pylori may predict clarithromycin resistance and eradication of infection at a high rate. J Clin Microbiol 2017;55:2400–5.
19. Mishra S, Singh V, Rao GR, Jain AK, Dixit VK, Gulati AK, et al. Detection of Helicobacter pylori in stool specimens: comparative evaluation of nested PCR and antigen detection. J Infect Dev Ctries 2008;2:206–10.
20. Noguchi N, Rimbara E, Kato A, Tanaka A, Tokunaga K, Kawai T, et al. Detection of mixed clarithromycin-resistant and susceptible Helicobacter pylori using nested PCR and direct sequencing of DNA extracted from faeces. J Med Microbiol 2007;56:1174–80.
21. Schabereiter-Gurtner C, Hirschl AM, Dragosics B, Hufnagl P, Puz S, Kovách Z, et al. Novel real-time PCR assay for detection of Helicobacter pylori infection and simultaneous clarithromycin susceptibility testing of stool and biopsy specimens. J Clin Microbiol 2004;42:4512–8.
22. Wiśniewska M, Nilsson HO, Bak-Romaniszyn L, Rechciński T, Bielański W, Płaneta-Małecka I, et al. Detection of specific Helicobacter pylori DNA and antigens in stool samples in dyspeptic patients and healthy subjects. Microbiol Immunol 2002;46:657–65.
23. Monteiro L, Gras N, Megraud F. Magnetic immuno-PCR assay with inhibitor removal for direct detection of Helicobacter pylori in human feces. J Clin Microbiol 2001;39:3778–80.
24. Hsin LJ, Chuang HH, Lin MY, Fang TJ, Li HY, Liao CT, et al. Laryngeal Helicobacter pylori infection and laryngeal cancer-case series and a systematic review. Microorganisms 2021;9:1129.
25. Godbole G, Mégraud F, Bessède E. Review: diagnosis of Helicobacter pylori infection. Helicobacter 2020;25:e12735.
26. Watanabe K, Nagata N, Nakashima R, Furuhata E, Shimbo T, Kobayakawa M, et al. Predictive findings for Helicobacter pylori-uninfected, -infected and -eradicated gastric mucosa: validation study. World J Gastroenterol 2013;19:4374–9.
27. Zheng W, Zhang X, Kim JJ, Zhu X, Ye G, Ye B, et al. High accuracy of convolutional neural network for evaluation of Helicobacter pylori infection based on endoscopic images: preliminary experience. Clin Transl Gastroenterol 2019;10:e00109.
28. Ono S, Dohi O, Yagi N, Sanomura Y, Tanaka S, Naito Y, et al. Accuracies of endoscopic diagnosis of Helicobacter pylori-gastritis: multicenter prospective study using white light imaging and linked color imaging. Digestion 2020;101:624–30.
29. Malfertheiner P, Megraud F, Rokkas T, Gisbert JP, Liou JM, Schulz C, et al. Management of Helicobacter pylori infection: the Maastricht VI/Florence consensus report. Gut 2022 Aug 8. https://doi.org/10.1136/gutjnl-2022-327745 [Epub ahead of print].
30. Liu WZ, Xie Y, Lu H, Cheng H, Zeng ZR, Zhou LY, et al. Fifth Chinese national consensus report on the management of Helicobacter pylori infection. Helicobacter 2018;23:e12475.
31. Binmaeil H, Hanafiah A, Mohamed Rose I, Raja Ali RA. Development and validation of multiplex quantitative PCR assay for detection of Helicobacter pylori and mutations conferring resistance to clarithromycin and levofloxacin in gastric biopsy. Infect Drug Resist 2021;14:4129–45.
32. Mégraud F, Floch P, Labenz J, Lehours P. Diagnostic of Helicobacter pylori infection. Helicobacter 2016;21(1 Suppl):8–13.
33. Helicobacter pylori Study Group, Chinese Society of Gastroenterology, Chinese Medical Association. 2022 Chinese national clinical practice guideline on Helicobacter pylori eradication treatment. Chin J Dig 2022;42:745–56.
34. Pohl D, Keller PM, Bordier V, Wagner K. Review of current diagnostic methods and advances in Helicobacter pylori diagnostics in the era of next generation sequencing. World J Gastroenterol 2019;25:4629–60.
35. Hulten KG, Genta RM, Kalfus IN, Zhou Y, Zhang H, Graham DY. Comparison of culture with antibiogram to next-generation sequencing using bacterial isolates and formalin- fixed, paraffin-embedded gastric biopsies. Gastroenterology 2021;161:1433–42.
36. Mishra S, Singh V, Rao GR, Dixit VK, Gulati AK, Nath G. Prevalence of Helicobacter pylori in asymptomatic subjects–a nested PCR based study. Infect Genet Evol 2008;8:815–9.
37. Redondo JJ, Keller PM, Zbinden R, Wagner K. A novel RT-PCR for the detection of Helicobacter pylori and identification of clarithromycin resistance mediated by mutations in the 23S rRNA gene. Diagn Microbiol Infect Dis 2018;90:1–6.
38. Marrero Rolon R, Cunningham SA, Mandrekar JN, Polo ET, Patel R. Clinical evaluation of a real-time PCR assay for simultaneous detection of Helicobacter pylori and genotypic markers of clarithromycin resistance directly from stool. J Clin Microbiol 2021;59:e03040-20.
39. Mégraud F, Lamouliatte H. Review article: the treatment of refractory Helicobacter pylori infection. Aliment Pharmacol Ther 2003;17:1333–43.
40. Kim SY, Park JM, Lim CH, Lee HA, Shin GY, Choe Y, et al. Types of 23S ribosomal RNA point mutations and therapeutic outcomes for Helicobacter pylori. Gut Liver 2021;15:528–36.
41. Vester B, Douthwaite S. Macrolide resistance conferred by base substitutions in 23S rRNA. Antimicrob Agents Chemother 2001;45:1–12.