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Contents

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World Journal of Clinical Cases

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Contents

World Journal of Clinical Cases

Semimonthly Volume 9 Number 1 January 6, 2021

Contents

World Journal of Clinical Cases

Semimonthly Volume 9 Number 1 January 6, 2021

Contents

ABOUT COVER

Editorial Board Member of *World Journal of Clinical Cases*, Dr. Antonio Corvino is a PhD in the Motor Science and Wellness Department of University of Naples "Parthenope". After obtaining his MD degree from the School of Medicine, Second University of Naples (2008), he completed a residency in Radiology at the University of Naples Federico II (2014). Following post-graduate training at the Catholic University of Rome, yielding a second level Master's degree in "Internal Ultrasound Diagnostic and Echo-Guided Therapies" (2015), he served on the directive board of Young Directive of Italian Society of Ultrasound in Medicine and Biology (2016-2018). His ongoing research interests involve ultrasound and ultrasound contrast media in abdominal and non-abdominal applications, mainly in gastrointestinal, hepatic, vascular, and musculoskeletal imaging. (L-Editor: Filipodia)

AIMS AND SCOPE

The primary aim of *World Journal of Clinical Cases* (*WJCC*, *World J Clin Cases*) is to provide scholars and readers from various fields of clinical medicine with a platform to publish high-quality clinical research articles and communicate their research findings online.

WJCC mainly publishes articles reporting research results and findings obtained in the field of clinical medicine and covering a wide range of topics, including case control studies, retrospective cohort studies, retrospective studies, clinical trials studies, observational studies, prospective studies, randomized controlled trials, randomized clinical trials, systematic reviews, meta-analysis, and case reports.

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The *WJCC* is now indexed in Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports/Science Edition, PubMed, and PubMed Central. The 2020 Edition of Journal Citation Reports® cites the 2019 impact factor (IF) for *WJCC* as 1.013; IF without journal self cites: 0.991; Ranking: 120 among 165 journals in medicine, general and internal; and Quartile category: Q3.

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META-ANALYSIS

Polymerase chain reaction-based tests for detecting *Helicobacter pylori* clarithromycin resistance in stool samples: A meta-analysis

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Abstract

BACKGROUND

Helicobacter pylori (*H. pylori*) infection is closely associated with the etiology of a variety of gastric diseases. The effective eradication of *H. pylori* infection has been shown to reduce the incidence of gastric carcinoma. However, the rate of *H. pylori* eradication has significantly declined due to its increasing resistance to antibiotics, especially to clarithromycin. Therefore, the detection of clarithromycin resistance is necessary prior to the treatment of *H. pylori*. Although many studies have been conducted on the use of polymerase chain reaction (PCR)-based tests to detect clarithromycin resistance in stool samples, no accurate data on the feasibility of these tests are available. Here, we performed a meta-analysis to assess the feasibility of these noninvasive tests.

AIM

To evaluate the reliability of PCR-based tests for detecting *H. pylori* clarithromycin resistance in stool samples.

METHODS

We searched PubMed, Medline, Embase, and other databases for articles that evaluated the value of the PCR analysis of stool samples for detecting the resistance of *H. pylori* to clarithromycin. We collected cross-sectional studies that met the inclusion criteria. Diagnostic accuracy measures were pooled using a random-effects model. The risk of bias was assessed using the Quality Assessment of Diagnostic Accuracy Studies-2 tool. Subgroup analysis was also conducted according to PCR type, purification technique, reference standard, mutation site, sample weight, number of patients, and age group, and the clinical utility of diagnostic tests was evaluated using the Likelihood Ratio Scatter Graph.

RESULTS

Out of the 1818 identified studies, only 11 met the eligibility criteria, with a total of 592 patients assessed. A meta-analysis of the random-effect model showed that PCR-based analysis of stool samples had high diagnostic accuracy for detecting

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clarithromycin resistance in patients infected with *H. pylori*. The combined sensitivity was 0.91 [95% confidence interval (CI): 0.83-0.95], Q = 30.34, and I^2 = 67.04, and the combined specificity was 0.97 (95%CI: 0.62-1.00), Q = 279.54, and I^2 = 96.42. The likelihood ratio for a positive test was 33.25 (95%CI: 1.69-652.77), and that for a negative test was 0.10 (95%CI: 0.05-0.18), with an area under the curve of 0.94. The diagnostic odds ratio was 347.68 (95%CI: 17.29-6991.26). There was significant statistical heterogeneity, and the sub-analyses showed significant differences in the number of patients, sample weight, purification methods, PCR types, mutation points, and reference standards. The included studies showed no risk of publication bias.

CONCLUSION

PCR-based tests on stool samples have high diagnostic accuracy for detecting *H. pylori* clarithromycin resistance.

Key Words: *Helicobacter pylori*; Clarithromycin resistance; Polymerase chain reaction; Feces; Meta-analysis

Core Tip: No consensus is available in the literature about the reliability of polymerase chain reaction (PCR)-based tests for detecting *Helicobacter pylori* (*H. pylori*) clarithromycin resistance in stool samples. This is the first meta-analysis deciphering these methods based on the numbers of true-positive, false-positive, false-negative, and true-negative test results. Our results show that PCR-based approaches on stool samples have high diagnostic accuracy for detecting *H. pylori* clarithromycin resistance.

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INTRODUCTION

Helicobacter pylori (H. pylori) is a gram-negative bacterium firstly identified from antral mucosa in 1984[1]. *H. pylori* is closely related to digestive diseases such as chronic gastritis, peptic ulcers, mucosa associated lymphoid tissue lymphoma and gastric carcinoma. The World Health Organization classified *H. pylori* as a group I carcinogen for stomach cancer, and its eradication therapy is highly recommended by the Kyoto global consensus[2]. Clarithromycin-based triple therapy encompassing a proton pump inhibitor and another antibiotic (amoxicillin or metronidazole) is generally conducted as the first-line treatment[3]. The eradication rate of *H. pylori*, however, has gradually decreased due to antibiotic resistance worldwide. In particular, clarithromycin resistance significantly increased from 13% in 2006-2008 to 21% in 2012–2016[4]. The latest clinical guidelines point out that clarithromycin triple therapy should be limited to patients that reside in areas with low *H. pylori* clarithromycin resistance[5,6]. Compared with previous empirical treatments for *H. pylori* eradication, tailored therapy including an antimicrobial susceptibility test leads to better outcomes[7]. The Maastricht IV/Florence Consensus Report suggests that susceptibility testing should be performed in regions where the clarithromycin resistance rate greater than 20%[8]. In addition, the Toronto Consensus recommends that susceptibility testing should be encouraged when patients undergo endoscopy[9]. Therefore, the detection of clarithromycin resistance is necessary prior to the treatment of *H. pylori*, especially in cases of refractory *H. pylori* infection. However, conducting susceptibility tests in all patients is currently impractical or impossible.

In the past, phenotypic methods have been widely used, including the broth dilution method, agar dilution method, disk diffusion tests, and E-test, all of which

were conducted using *H. pylori* isolated from patient stomach biopsy samples[10]. These methods have high accuracy, but the time-consuming nature, harsh conditions, and vulnerability to certain medicines caused by these methods limit their wide clinical application. The need for repeated endoscopy to obtain biopsies after failed therapy may render this approach cost-prohibitive. The emerging roles of genotypic methods have been recognized. For example, point mutations at specific loci of the 23S ribosomal ribonucleic acid (rRNA) were found to explain clarithromycin resistance in 1996[11]. Point mutations launch decreased affinity between ribosomes and clarithromycin so that the antibiotic is unable to interfere with bacterial protein biosynthesis[12]. Polymerase chain reaction (PCR)-based tests have been gradually acknowledged to evaluate the clarithromycin resistance by detecting 23S rRNA in *H. pylori* strains or biopsies[13-15]. However, this technique requires the use of a gastroscopy, which is particularly difficult for elderly and pediatric patients. PCR-based analysis of stool samples has received increasing attention for its noninvasiveness, convenience, and low cost. Studies have shown that the detection of clarithromycin resistance in feces by PCR has high accuracy[16,17], but some studies have suggested otherwise[18].

Although many studies have been conducted on the efficacy of PCR-based tests to detect clarithromycin resistance in stool, there are no data available on the reliability of these tests. Here, we performed a meta-analysis by collecting all useful data to assess the reliability of these noninvasive tests. This was the first meta-analysis to evaluate the reliability of PCR-based tests for detecting *H. pylori* clarithromycin resistance in stool samples.

MATERIALS AND METHODS

Protocol and registration

Our research was registered and approved on the International Prospective Register of Systematic Reviews, with the registration number CRD42019142429. We conducted this study following the recommendations of the Preferred Reporting Items for Systematic reviews and Meta-analyses[19].

Literature search

We searched PubMed, Medline, Embase, and other databases for related articles from January 1, 1987 through January 31, 2019 using the search terms "*Helicobacter pylori*", "*H. pylori*", "*Helicobacter* infection", "clarithromycin resistance", "antibiotic resistance", "feces", "polymerase chain reaction", and "PCR" with its Medical Subject Headings terms and keywords. Letters were also included if they could provide additional data. There were no restrictions on language and age in the literature search.

Study selection

Studies were eligible when they met the following criteria: (1) Observational studies about the detection of human clarithromycin resistance by PCR in feces without restrictions of language and age; (2) Patients infected with *H. pylori*; (3) Gold standard was provided; (4) Data could be extracted; (5) Full text was available; and (6) Letters could be included if they could provide the information required for studies to be included.

Studies were excluded if they were abstracts, reviews, case reports, or animal experiments or if they could not provide useful data.

Data extraction and quality evaluation

All the data were extracted from eligible full-text studies by two investigators (RJG and HL) independently. The data included the first author, year of publication, number of patients, country, age groups, gender, method used to diagnose *H. pylori*, sample weight, purification methods, PCR types, point mutations, reference standard, and diagnostic study data.

Two investigators (RJG and HL) independently assessed the quality of each study using the QUADAS-2 tool, which was designed to assess the quality of primary diagnostic accuracy studies included in the article.

The tool includes four important areas: Patient selection, index tests, reference standard, flow, and timing[20]. Using this tool, the risk of bias was judged as "low", "high", and "unclear". If all the signaling questions for an area were answered with "yes", then the risk of bias was considered "low". If any signaling questions were

answered with "no", then this could indicate potential bias. The level of agreement on article quality was generally high (> 80% crude agreement, kappa = 0.65). Funnel plots were used to evaluate the risk of publication bias. Any divergence was resolved by a third reviewer (XML).

Statistical analysis

The meta-analysis was conducted using stata15.0 software (College Station, TX, United States). A random-effect model was used in all the analyses due to heterogeneity. The diagnostic accuracy indexes used in the analysis were pooled sensitivity, pooled specificity, positive likelihood ratio, negative likelihood ratio, diagnostic odds ratio, and summary receiver operating characteristics curve. *I*-squared statistic and *Q* test were used to assess heterogeneity. Deeks' funnel plot asymmetry test was used for publication bias, and slope coefficient *P* values < 0.05 indicated significant asymmetry. We also performed a subgroup analysis to assess whether PCR type, purification technique, reference criteria, mutation site, sample weight, number of patients, and age group affected the pooled estimates.

RESULTS

Study selection

From the 1818 initial articles, 11 studies enrolling a total of 592 participants were finally included. The study selection process is shown in Figure 1.

Characteristics of the included studies

The characteristics of all the included studies are shown in Table 1; the data extracted by the two investigators (RJG and HL) were identical. A total of 11 studies with 592 participants were included in the meta-analysis. These studies were conducted in seven countries, and all the studies were published in English. All the eligible studies were published between 2004 and 2017. The sample sizes ranged from seven to 125. The patients enrolled included adults and children. Most of the studies did not provide accurate data about the male-female ratios and age groups. There was variation in the type of PCR used in the different studies. Nested PCR was used in five studies (45%)[16,21-24]; real-time PCR and Genotype were used in five studies[17,25-28] and one study (10%)[18], respectively. The reference standards for clarithromycin resistance were different: Minimum inhibitory concentration in nine studies (82%) and PCR-based test of biopsy samples in two studies (18%). The gene mutation loci detected were A2142 and A2143 in 10 studies (91%) and A2143, A2142 and A2717 for one study (9%). Key data were successfully extracted from all the studies, including the number of true positives, false positives, false negatives, and true negatives.

Risk of bias within studies

The quality assessment scores of more than 50% of the articles indicated that the risk of bias was low. Figure 2 visually shows the risk of bias estimated for all of the included studies.

Pooled estimate for the stool PCR-based test

The results of the analysis are shown in Figure 3-6. The pooled sensitivity was 0.91 [95% confidence interval (CI): 0.83-0.95], and the pooled specificity was 0.97 (95%CI: 0.62-1.00) (Figure 3). The positive likelihood ratio was 33.25 (95%CI: 1.69-652.77), and the negative likelihood ratio was 0.10 (95%CI: 0.05-0.18) (Figure 4) with an area under the curve (Figure 5) of 0.94. The diagnostic odds ratio was 347.68 (95%CI: 17.29-6991.26) (Figure 6). There was significant statistical heterogeneity; the I^2 values for sensitivity and specificity were 67.04 and 96.42, respectively, with a statistically significant *Q* test result ($P < 0.05$).

Subgroup analysis

The significant heterogeneity could be explained by clinical and methodological variation. We used stata15.0 software to conduct regression and subgroup analyses to determine the source of heterogeneity based on these variables. The results indicated that sample weight, purification methods, PCR types, mutation points, and reference standards accounted for the heterogeneity in the pooled sensitivity and specificity, and the number of patients only explained the heterogeneity of the pooled sensitivity (Figure 7).

Table 1 Baseline characteristics of included studies

Ref.	Country	Diagnose *H. pylori*	Patient number	Source	Gender, M/F	Sample weight, mg	Purification	PCR type	Point mutations	Gold standard
Fontana *et al*[21], 2003	Italy	Culture	125	Adult + child	NA	220	Qiagen	Nested	A2143, A2142, A2717	MIC > 1 μg/mL
Noguchi *et al*[22], 2007	Japan	UBT	98	Adult	NA	50	Promega	Nested	A2142, A2143	MIC > 1 μg/mL
Rimbara *et al*[23], 2009	Japan	NA	50	Adult	24/26	50	Promega	Nested	A2142, A2143	MIC > 1 μg/mL
Lottspeich *et al*[25], 2007	Germany	Histo; Culture; UBT; HpSA	46	Child	NA	200	Qiagen	RT	A2142, A2143	MIC > 1 μg/mL
VÉCSEI *et al*[26], 2010	Austria	RUT; Histo; Culture	67	Child	NA	200	Qiagen	RT	A2142, A2143	MIC > 1 μg/mL
Scaletsky *et al*[27], 2011	Brazil	Culture; Histo; RUT	45	Child	NA	200	Qiagen	RT	A2142, A2143	MIC > 1 mg/L
Rimbara *et al*[24], 2005	Japan	HpSA; Culture	7	NA	NA	NA	Q-BIOgene	Nested	A2142, A2143	MIC > 1 μg/mL
Giorgio *et al*[28], 2016	Italy	UBT	52	Adult	23/29	300	THD fecal test	RT	A2142, A2143	PCR in biopsy
Brennan *et al*[18], 2016	Ireland	UBT; RUT	17	Adult	NA	NA	PSP spin stool	Genotype	A2146, A2147	PCR in biopsy
Osaki *et al*[16], 2017	Japan	HpSA	40	Adult	NA	200	DNA Plus Kit	Nested	A2142, A2143	MIC > 0.5 mg/L
Schabereiter-Gurtner *et al*[17], 2004	Austria	Histo; RUT;Culture	45	Adult	NA	200	Qiagen	RT	A2142, A2143	MIC ≥ 1 μg/mL

F: Female; *H. pylori*: *Helicobacter pylori*; Histo: Histopathology; HPSA: *Helicobacter pylori* serum antigen; M: Male; MIC: Minimal inhibitory concentration; NA; Not available; PCR: Polymerase chain reaction; RT: Real-time polymerase chain reaction; RUT: Rapid urea test; UBT: Urea breath test.

Risk of publication bias

Deeks' funnel plot test (Figure 8) indicated that the risk of publication bias was not significant (P = 0.22).

Clinical utility of a diagnostic test

The clinical utility of a diagnostic test was evaluated using the Likelihood Ratio Scatter Graph (Figure 9). The likelihood ratio summation points in the upper left quadrant were a function of mean sensitivity and specificity, indicating that the test was useful for confirming the presence and absence of disease. Specifically, this figure shows the summary points of the likelihood ratio in the upper left quadrant, which was a function of the mean sensitivity and specificity.

DISCUSSION

This study is the first meta-analysis to assess the reliability of detecting clarithromycin resistance in the feces of patients with *H. pylori* by PCR-based tests. Eleven studies were included to evaluate the clinical application value of the test. The meta-analysis results showed that the technique has a high diagnostic accuracy for detecting clarithromycin resistance in patients infected with *H. pylori*. The heterogeneity can be explained by the number of patients, sample weight, purification method, PCR type, mutation point, and reference standard, and the variation in the methodological quality of the included studies may also lead to heterogeneity. The included studies had no risk of publication bias. A likelihood ratio scatter graph revealed that the test was useful for confirming the presence and absence of the disease. A previous study[18] reached a different conclusion, namely, that the Genotype HelicoDR assay was unsuitable for the accurate detection of clarithromycin resistance in stool specimens.

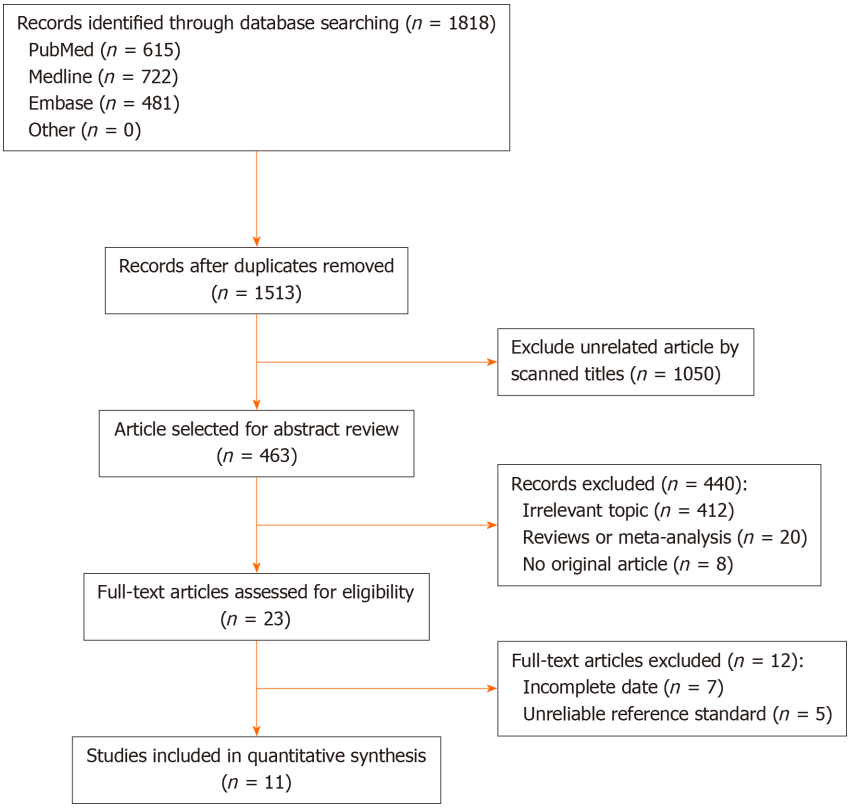

Figure 1 Study selection process.

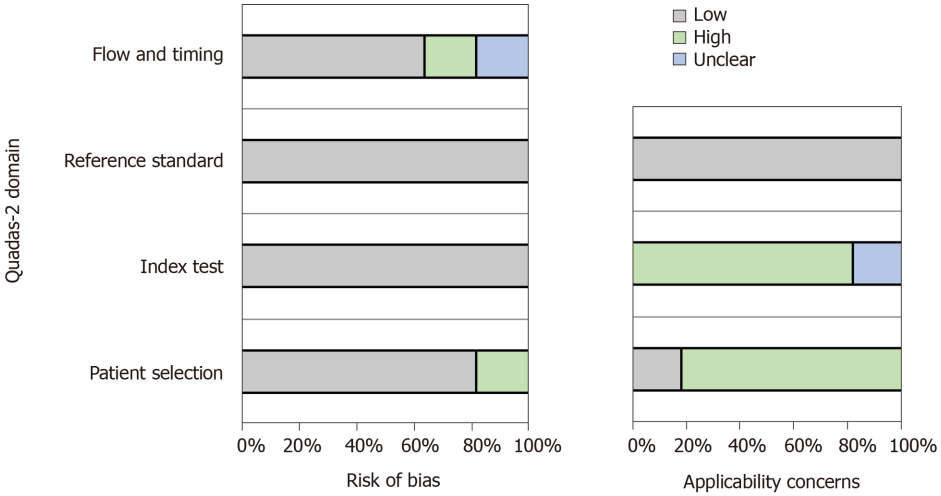

Figure 2 Risk of bias assessment.

This considerable discrepancy may arise for several reasons in pre-analysis and laboratory practice. Improper storage/transportation and repeated freezing/thawing of stool samples may impair test sensitivity due to enzymatic or mechanical degradation of DNA, and the presence of a large number of diverse symbiotic bacteria in feces may hinder the specificity of detection. Due to our overall data analysis, we think that the PCR-based tests still have high diagnostic accuracy.

H. pylori has been reported to be closely related not only to diseases of the digestive system but also to cardiovascular, vascular, and autoimmune diseases. The Kyoto global consensus recommends that *H. pylori* infection should be efficiently treated to prevent more severe complications. The clarithromycin-based standard triple therapy has been recommended by the Maastricht Treaty consensus to eradicate *H. pylori* in children and adults[8]. However, the rate of *H. pylori* eradication has decreased annually, primarily as a result of the development of resistance to antibiotics, such as

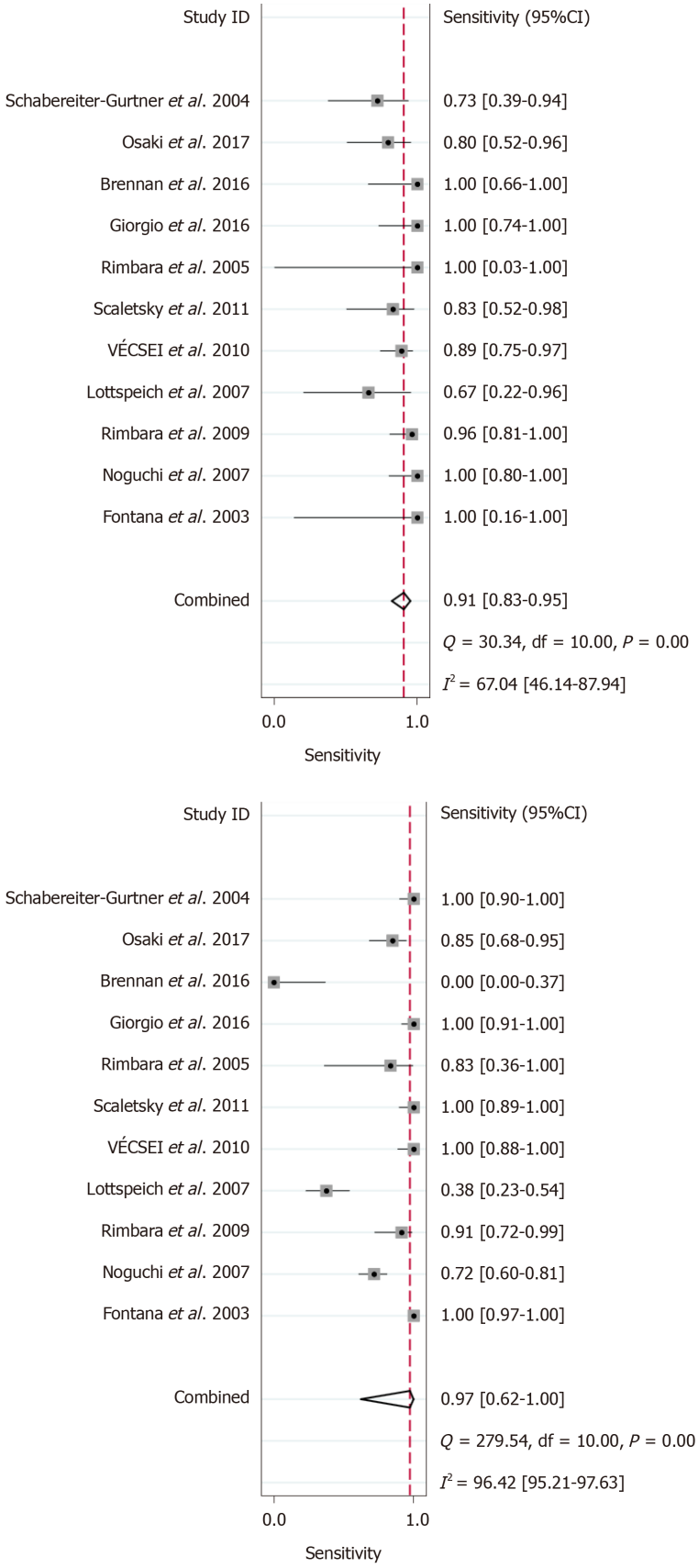

Figure 3 Overall sensitivity and specificity. CI: Confidence interval.

clarithromycin[11]. Furthermore, *H. pylori* infection is frequently acquired during childhood[29]. Some studies have shown that the eradication of *H. pylori* may benefit children with nonnuclear dyspepsia[30,31], and that there are unexpectedly higher rates of clarithromycin-resistant *H. pylori* in younger age groups[32,33]. However, gastroduodenal endoscopy is not generally advisable for the pediatric population. Thus, developing simple and noninvasive means to diagnose antibiotic susceptibility will greatly facilitate antibiotic therapy[34]. Susceptibility testing should be carried out in regions with high clarithromycin resistance[8] or in patients who are not suitable for gastroscopy or have refractory infections, as susceptibility testing can avoid the hazards associated with the abuse of antibiotics and prevent increases in the development of secondary or multiple antibiotics resistance.

The application of phenotypic detection technology is limited by its time-consuming nature, demanding conditions, and vulnerability to certain drugs. In addition, traditional PCR tests specimens, including biopsies, isolates, and gastric juices, that require invasive testing. PCR-based tests usually allow for both the detection of *H. pylori* and clarithromycin susceptibility simultaneously. The former detects specific genes, such as 23S rRNA, to confirm *H. pylori* infection, which has been proved to have high diagnostic accuracy[35,36]. Specific point mutation functional domains of the 23S rRNA gene usually lead to clarithromycin resistance, which is most frequently located in domain V and domain VI.

In a real clinical setting, the novel fecal PCR-based test has many advantages compared with traditional methods. First, patients only need to provide a small amount (nearly 200 mg) of fresh stool samples rather than undergo gastroscopy. Although special storage containers may be required, the results will be communicated to patients within hours, which certainly facilitates treatment decisions. Furthermore, the cost of fecal PCR is clearly lower than conventional methods for assessing antimicrobial resistance, and no additional expenses of general anesthesia and hospital admission for gastroscopy are needed. This approach could also be used with a wider range of people, including elderly and pediatric patients, and would thus be feasible for clinical application in small- and medium-scale hospitals in developing countries. In addition, this technique can not only provide accurate gene-level information before eradication but also meets the requirements for noninvasive testing for posttreatment follow-up examinations. Resistance to other antibiotics can also be predicted. For example, the rdxA and frxA genes are associated with metronidazole resistance[37,38], 16S ribosomal DNA mutations participate in tetracycline resistance[39], and the role of the gyrA gene in fluoroquinolone-resistant strains has been determined[40].

CONCLUSION

Overall, PCR-based analysis of stool samples has high diagnostic accuracy for detecting clarithromycin resistance in patients infected with *H. pylori*. The advantages of these tests include its noninvasiveness, convenience, and low cost compared with traditional detection methods. Therefore, this method could be help improve the eradication rate of *H. pylori*, especially in regions showing high resistance to clarithromycin.

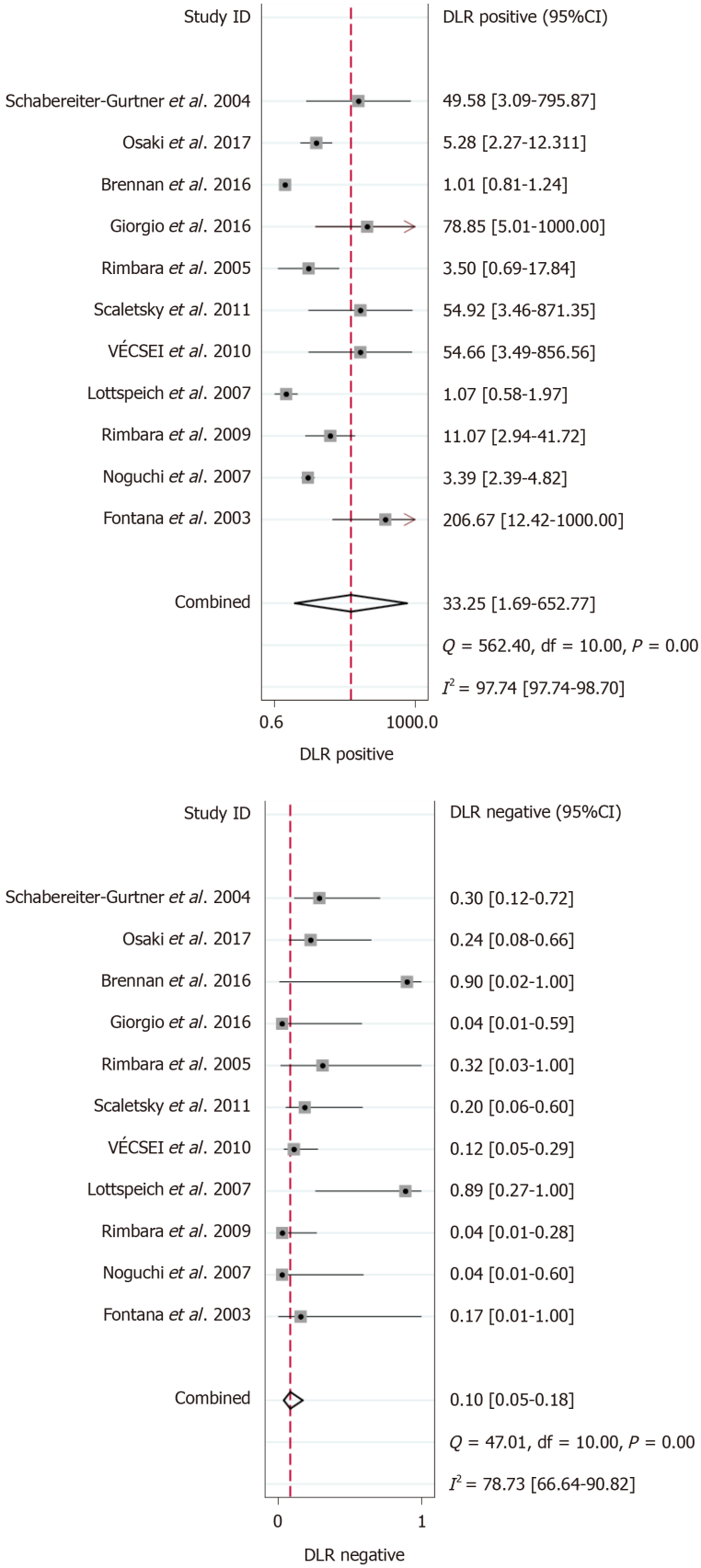

Figure 4 Overall likelihood ratio for positive and negative tests. CI: Confidence interval.

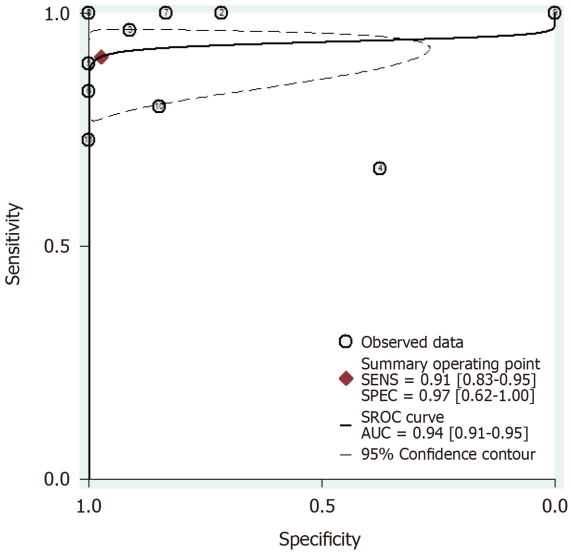

Figure 5 Summary receiver operating characteristics curve based on sensitivity and specificity. AUC: Area under the curve; SROC: Summary receiver operating characteristics.

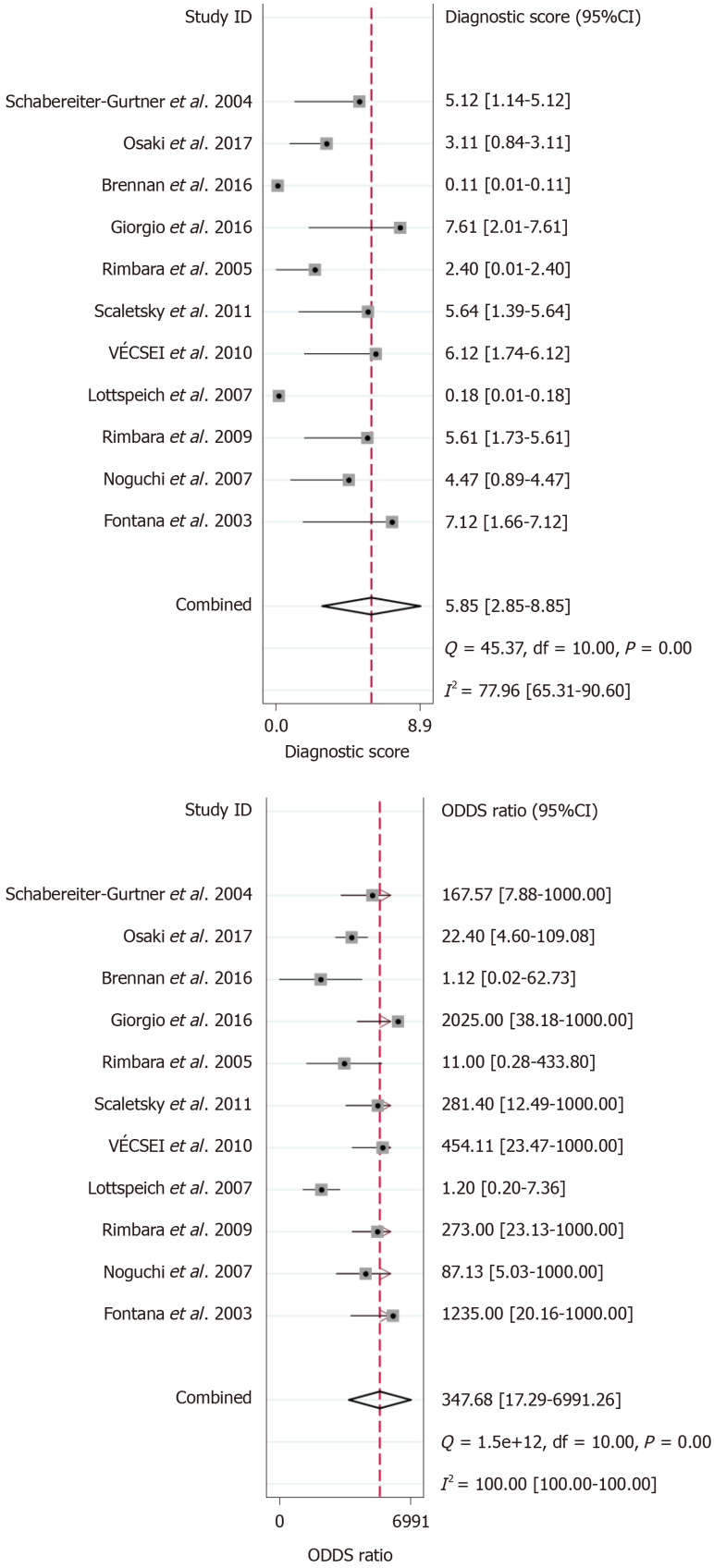

Figure 6 Diagnostic odds ratio. CI: Confidence interval.

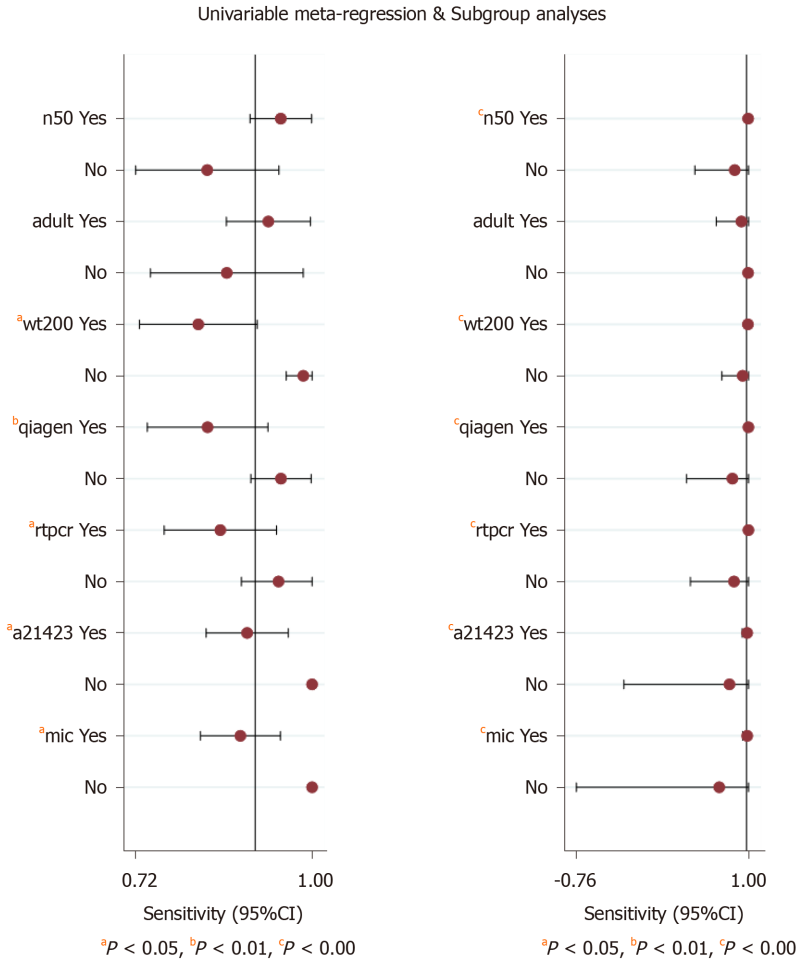

Figure 7 Univariable meta-regression and subgroup analysis. a21423: A2142 and A2143; CI: Confidence interval; mic: Minimal inhibitory concentration; n50: The number of patients was 50; wt200: Sample weight was 200 mg.

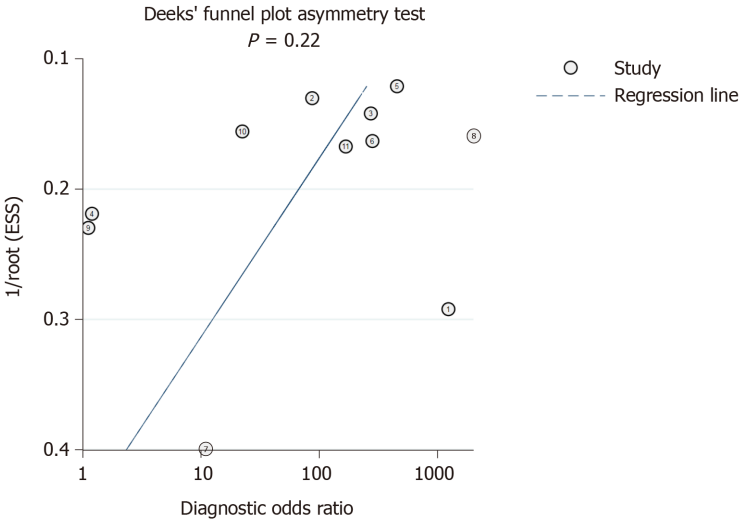

Figure 8 Deeks' funnel plot.

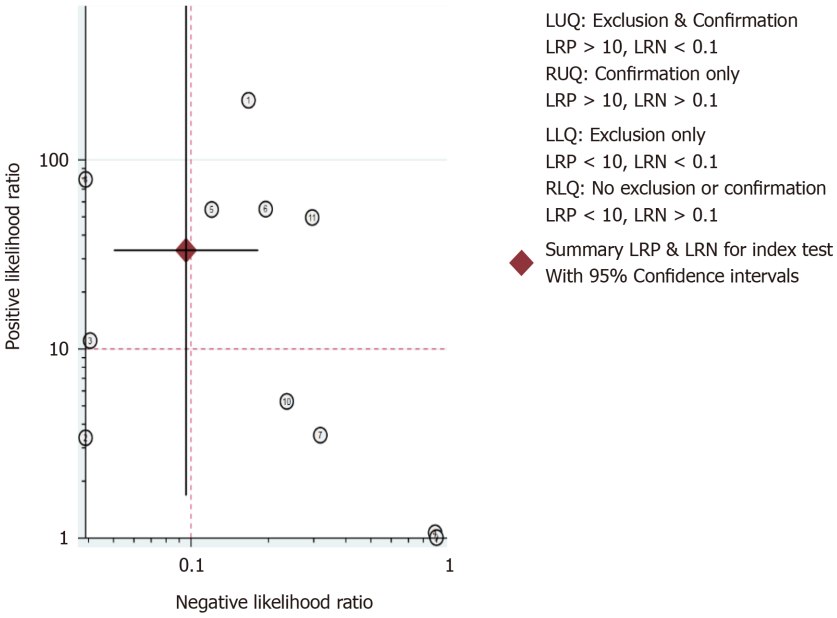

Figure 9 Likelihood ratio scatter graph.

ARTICLE HIGHLIGHTS

Research background

The eradication rate of *Helicobacter pylori* (*H. pylori*) is gradually decreasing due to antibiotic resistance worldwide, in particular clarithromycin resistance.

Research motivation

The detection of clarithromycin resistance is necessary prior to the treatment of *H. pylori*, accurate data on the feasibility of stool polymerase chain reaction (PCR)-based tests are not available.

Research objectives

We performed a meta-analysis to assess the feasibility of PCR-based tests for detecting *H. pylori* clarithromycin resistance in stool samples.

Research methods

We collected cross-sectional studies that met the inclusion criteria. This is the first meta-analysis based on true-positive, false-positive, false-negative, and true-negative test results.

Research results

A meta-analysis of the random-effect model showed that PCR-based analysis of stool samples had high diagnostic accuracy for detecting clarithromycin resistance in patients infected with *H. pylori*.

Research conclusions

PCR-based tests on stool samples have high diagnostic accuracy for detecting *H. pylori* clarithromycin resistance.

Research perspectives

This non-invasive, convenient, and inexpensive method can increase the eradication rate of *H. pylori*, especially in areas with high clarithromycin resistance.

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