Gastric Juice-Based Genotypic Methods for Diagnosis of Helicobacter pylori Infection and Antibiotic Resistance Testing: A Systematic Review and Meta-analysis

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ABSTRACT

Objective: *To evaluate the diagnostic efficacy of gastric juice–based genotypic methods for* Helicobacter pylori *detection and antibiotic resistance testing.*

Methods: *We used electronic databases including MEDLINE, EMBASE, Web of Science, and the Cochrane Central Register of Controlled Trial for literature survey using keywords such as "gastric juice," "Helicobacter pylori," and their synonyms. The quality of the studies was assessed using QUADAS-2. Summary performance measures (sensitivity, specificity, positive predictive values, negative predictive values, diagnostic odds ratio, and area under the summary receiver-operating characteristic curve) and HSROC curves were produced. In addition, Fagan plots were applied to illustrate the relationship among the prior test probability, PLR/NLR, and posterior test probability.*

Results: *Our study cohort comprised eight studies with 1235 participants (617 participants with* H. pylori infection *and 618 participants with non-*H. pylori *infection). Pooled sensitivity and specificity with a corresponding 95% CI of gastric juice–based genotypic methods reflected values of 94% (95% CI, 86%-98%) and 98% (95% CI, 85%-100%), respectively. The global sensitivity and specificity of clarithromycin resistance were 92% (95% CI, 85%-96%) and 90% (95% CI, 80%-95%), respectively.*

Conclusion: *Gastric juice–based genotypic methods can be used for diagnostic prediction of* H. pylori *infection as well as clarithromycin resistance testing.*

Keywords: *Helicobacter pylori, gastric juice, meta-analysis*

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a gram-negative and microaerophilic bacterium that colonizes the gastric mucosa.[1] It is widely accepted that *H. pylori* is a major cause of gastritis and peptic ulcers, as well as atrophy, intestinal metaplasia, intraepithelial neoplasia, and mucosa-associated lymphoid tissue lymphoma (MALT).[1,2] Globally ≥50% of individuals are infected, and the prevalence of *H. pylori* infection is higher in developing countries.[2]

Proton pump inhibitors (PPIs) in combination with antibiotics have been commonly used for the treatment of *H. pylori* infection. In recent years, PPI triple therapies (PTTs), as well as Bismuth-containing quadruple therapy (BCQT), have been recommended as treatment regimens.[2] However, eradication rates have decreased to ~70% or lower[2] for PTT and 70%-94% for BCQT. Antibiotic resistance, particularly toward clarithromycin and metronidazole, is accepted to be one of the major causes for this decline in treatment efficacy.[3,4] Accordingly, individual treatments based on antibiotic sensitivity testing was proposed as a new alternative to further improve the efficacy of *H. pylori* infection eradication and the value and necessity of this treatment modality have been discussed widely. Antibiotic resistance testing, mainly including phenotypic and genotypic methods, played a key role in such strategies. Phenotypic methods consisting of agar dilution experiments, disk diffusion tests, and epsilometer tests (E-test) were viewed as the "gold standard" of antibiotic resistance testing.[5] However, shortcomings such as the requirement of strict testing conditions and a time-consuming process limited its clinical use.[6] Genotypic methods, mainly including histology-based polymerase chain reaction (PCR), reverse transcription-polymerase chain reaction (RT-PCR), and PCR-RFLP (polymerase chain reaction-restriction

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fragment length polymorphism) techniques, aimed to detect point mutation genes of 23S rRNA,[7] rdxA and frxA genes,[8] and 16S ribosomal DNA[9] as manifestations of antibiotic resistance. Genotypic techniques were effective in testing antibiotic resistance within a shorter duration and using a single step without *H. pylori* culture as compared to phenotypic methods. However, there were limitations such as an uneven distribution of *H. pylori* on gastric mucosa and injury of gastric mucosa as well.[10]

In recent years, several studies have explored the potential of gastric juice–based genotypic methods in accurately detecting *H. pylori* infection as well as antibiotic(s) susceptibility.[10-18] Genotypic methods using gastric juice were adopted to detect *H. pylori* infection and test antibiotic resistance with variable results on parameters like sensitivity and specificity. Based on these studies, we aimed to systematically review the current status of gastric juice–based genotypic methods and further evaluate the diagnostic efficacy of *H. pylori* detection and antibiotic resistance testing.

MATERIALS AND METHODS

Search Strategy

We performed a systemic literature search in the databases of MEDLINE, EMBASE, Web of Science, and Cochrane Database from the date of inception of database till December 30 2019 according to the established protocol. Academic journals, dissertations, and conference proceedings were included irrespective of gray literature status. The key words for literature search included "Helicobacter pylori," "gastric juice," "genotypic method," and their synonyms. The search criteria and strategies for electronic databases are listed in Table 3 with the example of PubMed. The reference lists of the studies were also searched for potentially relevant titles. The study was conducted in accordance with the standards set forth by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.[19]

Table 3. Searching Strategy with Example of PubMed

#1 Helicobacter pylori[MeSH] OR Helicobacter pylori[abstract/title] OR *H. pylori*[abstract/title]
#2 "gastric juice"[abstract/title] OR "gastric juice"[MeSH]
#3 Genotypic[title/abstract] OR resistance[title/abstract] OR PCR[title/abstract] OR polymerase chain reaction[title/abstract] OR RT-PCR[title/abstract] OR PCR-RFLP[title/abstract] OR genotyping[title/abstract] OR susceptibility[title/abstract]
#4 #1 AND #2 AND #3

Inclusion and Exclusion Criteria

The inclusion criteria for the studies were as follows: (i) The participants should be patients with diagnosis/excluded diagnosis of *H. pylori* infection; (ii) The reference standard of *H. pylori* infection should be a combination of at least two of the following diagnostic tests, that is, $^{13}C/^{14}C$ urease breath test (UBT), *H. pylori* histology, rapid urease test (RUT), and *H. pylori* culture, and the reference standard of antibiotic resistance testing should be phenotypic methods including agar dilution experiments, disk diffusion tests, and E-test; (iii) The diagnostic test to be evaluated should be gastric juice–based genotypic methods (including PCR, RT-PCR, and PCR-RFLP techniques) for *H. pylori* infection and antibiotic resistance testing; (iv) the studies should provide complete data of sufficiently constructed 2 × 2 contingency tables for further meta-analysis calculations; (v) reports on retrospective or prospective observational studies.

The exclusion criteria for the studies were as follows: (i) duplicate articles which evaluated the same sample; (ii) animal experiments; (iii) case reports; (iv) reviews; (v) meta-analysis.

Qualitative Assessment of Studies

Two reviewers (Xiao-Bei Si and De-Ying Bi) independently assessed the quality of each eligible study using the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2).[20] The Review Manager (version 5.3, Nordic Cochrane Centre, the Cochrane Collaboration, Copenhagen, Denmark) was adopted to generate figures showing quality assessment results. QUADAS-2 offers a significantly improved tool for distinguishing between bias and applicability. Every study was qualified based on four key domains namely patient selection, index test, reference standard, and flow and timing to be accurate. Each domain was assessed in terms of the risk of bias according to the signaling questions with scores such as "yes" (for reported), "no" (for not reported), or "unclear" (for inadequate information to make a judgment).

Data Extraction

All retrieved reports were screened by two reviewers (Xiao-Bei Si and Shuo Zhang) independently. Titles and abstracts were scrutinized in all the relevant articles. Full text screening was performed for further assessment following the inclusion and exclusion criteria.

Disagreements were resolved by discussion or by external consultation.

Two reviewers (Xiao-Bei Si and Lin-Yu Huo) independently extracted data from the included studies. The data included authors, publication year, sample size, reference standard, target genes, and diagnostic results (e.g., true positive number, false positive number, true negative number, and false negative number). Data were extracted in a specific format.

Publication Bias Assessment

We used Stata statistical software (version 15.0, StataCorp LP, College Station, TX, USA) to perform a quantitative analysis of all publication bias as detected by funnel plots and the Deek's test. Potential existence of publication bias was reflected in asymmetric distribution of data points in the funnel plot with a quantified significance value of $P < .05$.

Statistical Analysis

A bivariate random-effects model was adopted to calculate the pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), and area under the summary receiver-operating characteristic curve (AUC). Then, the hierarchical summary receiver-operating characteristic (HSROC) curves were constructed to assess the overall diagnostic performance using hierarchical logistic regression.[21] The summary sensitivity and specificity points were presented along with a 95% confidence region and a 95% prediction region.

We explored the heterogeneity among studies through visual examination of the forest plot and HSROC curve in accordance with Cochrane Collaboration's guidelines.[21] Where appropriate, threshold analysis, subgroup analysis, and meta-regression analysis were performed to explore the sources of heterogeneity. The Spearman correlation coefficient between the logit of sensitivity and the logit of 1-specificity was computed to assess the threshold effect. A strong positive correlation would suggest a threshold effect of $P < .05$. The planned variables for subgroup analysis and meta-regression analysis were target genes, participant number, publication year, and publication types. Fagan plots were applied to illustrate the relationship among prior test probability, PLR/NLR, and posterior test probability. All statistical analyses were performed with Stata statistical software (version 15.0, StataCorp LP, College Station, TX, USA).

RESULTS

Literature Search and Study Selection

We identified 516 records using our established search strategy. Of these, 324 were excluded as duplicated records, 131 were unrelated articles, and 42 were non-clinical trials. We subjected 19 studies to full-text screening. A total of 10 studies were excluded due to reference standards that did not meet the inclusion criteria. One study[18] was excluded because it was not fit for meta-analysis due to incomplete outcomes reported. A flow chart of the article screening and selection processes is shown in Figure 1.

Study Design and Patient Characteristics

A final set of 8 studies[10-17] with 1235 participants (617 *H. pylori*–infected and 618 non-*H. pylori*–infected individuals) were included. The sample population of each study ranged from 34 to 547. There was no record of children being enrolled. All studies were published in English. One study[11] was published as an abstract. One study[17] was published as a letter to editors. The rest were published as full text articles. The characteristics for including studies are shown in Table 1. The quality assessment of the included studies for *H. pylori* infection and antibiotic resistance testing were accomplished through QUADAS questionnaire, and the results are shown in Supplementary Figures 1 and 2.

Publication Bias

The Deek's funnel plot revealed that the slope coefficient for *H. pylori* infection and antibiotic resistance testing were 0.75 and 0.69 respectively, suggesting non-existence of significant publication bias in this meta-analysis (Supplementary Figure 3).

Diagnosis of H. pylori Infection

Totally 7 studies[10-16] involved analysis of diagnostic accuracy of *H. pylori* infection. The pooled sensitivity and specificity were 94% (95% CI, 86%-98%) and 98% (95% CI, 85%-100%) with forest plots showing in Figure 2. PLR, NLR, DOR, and AUC showed values of 46.54 (95% CI, 5.81-372.52), 0.06 (95% CI, 0.02-0.15), 802.25 (95% CI, 62.52-10294.24), and 0.96 (95% CI, 0.94-0.98).

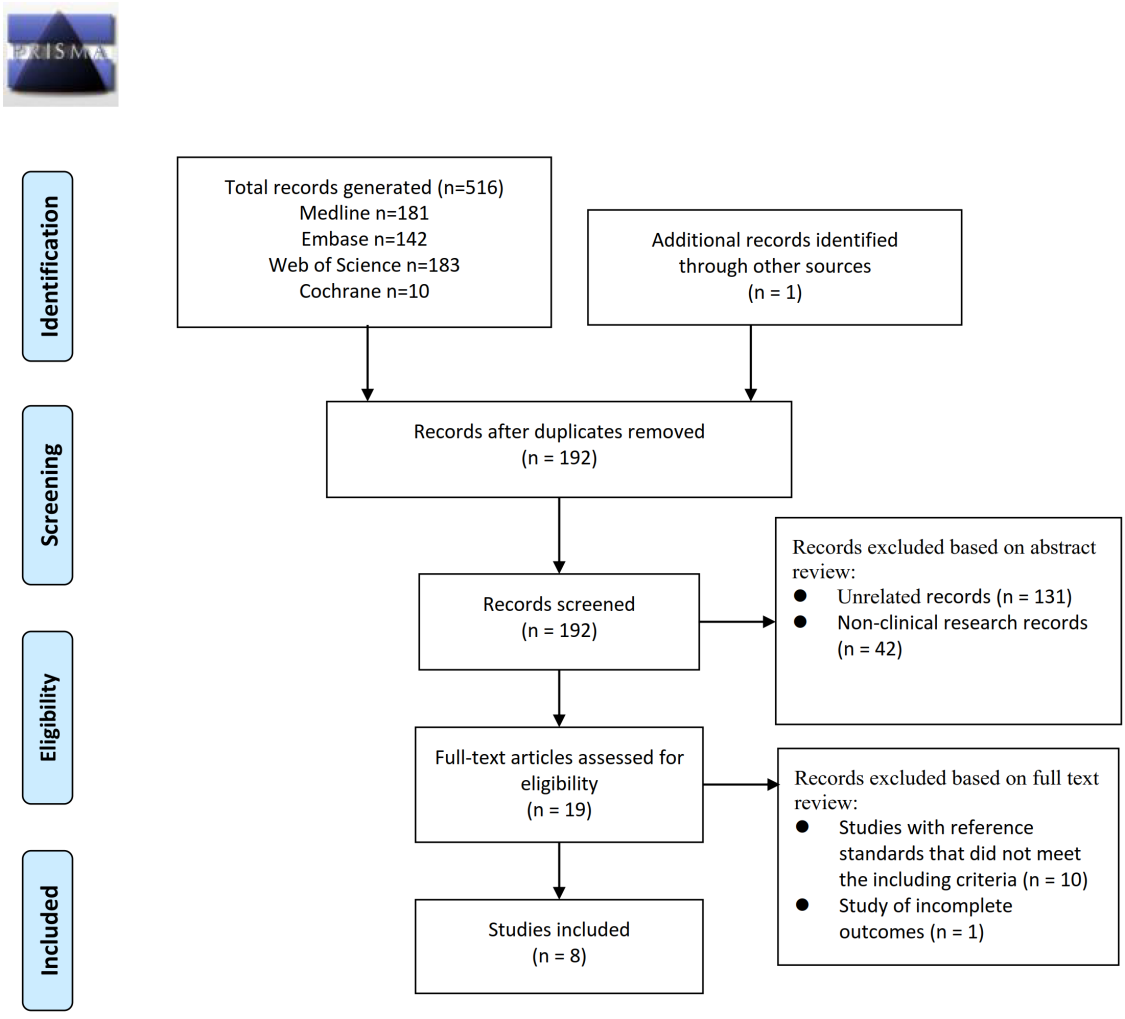

Figure 1. Flow-chart for articles identified and analyzed in this meta-analysis.

Figure 3 shows the HSROC curves for diagnosis of *H. pylori* infection along with the summary point of sensitivity and specificity, as well as 95% confidence region and 95% prediction region. As a result, the HSROC curve for diagnosis of *H. pylori* infection is seen approaching the top left-hand corner of the graph with a wide area of both confidence region and prediction region, indicating high accuracy with high between-study heterogeneity and lack of precision.

Figure 2 showed heterogeneity among studies as well, in particular for specificity. Spearman analysis showed that there was no threshold effect within the included studies (r = 0.37, P = .14). Further subgroup analysis and meta-regression indicated that participant number (>100 vs. <100, P < .01), publication year (<2010 vs. ≥2010, P < .01), and publication types (full text vs. non-full text, P < .001) significantly contributed to the heterogeneity in sensitivity (Table 2 and Figure 4). In addition, it was not appropriate to add target genes as covariate for subgroup analysis because varied types of genes or combined genes were adopted in the studies included.

Diagnosis of Antibiotics Resistance

Four studies[10,13,14,17] involved analysis of diagnostic accuracy of clarithromycin resistance by comparing with a reference standard of E-test and agar dilution experiments. The pooled sensitivity and specificity were 92% (95% CI, 85%-96%) and 90% (95% CI, 80%-95%) with forest

Table 1. Summary of Including Studies

Study	*N*	Ages of Including Participants (years)	Inclusion and Exclusion Criteria of Participants	Reference Standard of *H. pylori* Infection	Reference Standard of ART	Target Genes	Other *H. pylori* Detection Meanings
Peng et al. 2017[10]	178	Mean ± SD: 41.6 ± 12.8 years Range: from 19 to 68 years	Including criteria: patients with the complaint of dyspepsia Exclusion criteria: patients who received any *H. pylori* eradication therapy, including antibiotics and acid-suppressive drugs (PPIs, H2-receptor antagonists, bismuth agent, or antacids)	Positive: both positive for RUT strongly positive (becoming red within 2 minutes) and histology test (Warthin-Starry silver staining) positive Negative: both negative for RUT (no color change within 2 hours) and histology	E-test	Cag H gene for *H. pylori* infection; A2142G and A2143G mutants of the *H. pylori* 23S rRNA gene for CRT	*H. pylori* culture
Chandrasakha et al. 2014[11]	64	Mean ± SD: 59.61 ± 15.80 Range: from 15 to 80 years	Inclusion criteria: patients who presented with upper gastrointestinal bleeding Exclusion criteria: not mentioned	*H. pylori* infection was defined to be positive on the basis of a positive culture or a positive rapid urease test plus histopathology	NA	23SrRNA gene for *H. pylori* infection	*H. pylori* culture; RUT; Histology
Datta et al. 2005[12]	45	Range: from 21 to 60 years	Inclusion criteria: ① presence of duodenal or gastric ulcer/gastric adenocarcinoma/ non-ulcer dyspepsia; ② age between 21 and 60 years Exclusion criteria: ① previous anti-*H. pylori* therapy; ② pregnancy and lactation; and (iii) alcoholism, non-steroidal anti-inflammatory drug intake	Positive: ① positive for histology; ② positive for both RUT and culture; Negative: all negative for RUT, histology, culture	NA	urease B gene for *H. pylori* infection	*H. pylori* culture; RUT; Histology
Hsieh et al. 2019[13]	547	Mean ± SD: 55 ± 13 (Tx naive) 61 ± 14 (post-1st treatment) 57 ± 12 (post-2nd treatment) 58 ± 13 (post-3rd treatment)	Including criteria: patients who presented with dyspepsia; Exclusion criteria: current ingestions of antibiotics, bismuth or proton-pump inhibitor (PPI) within the prior four weeks; previous gastric operations, severe underlying comorbidity (decompensated liver cirrhosis and end-stage renal disease), and pregnant women	*H. pylori* infection was defined to be positive according to the following clinical gold standards: (a) concomitant positive results of both histology and rapid urease test; OR (b) one positive result from either histology or rapid urease test plus positive UBT result	E-test	Combined detection of urease A gene and Cag A gene for H pylori infection A2142G and A2143G mutants of the *H. pylori* 23S rRNA gene for CRT	*H. pylori* culture

Study	*N*	Ages of Including Participants (years)	Inclusion and Exclusion Criteria of Participants	Reference Standard of *H. pylori* Infection	Reference Standard of ART	Target Genes	Other *H. pylori* Detection Meanings
Kuo et al. 2015[14]	268	NA	Inclusion criteria: patients with the complaint of dyspepsia Exclusion criteria: ingestion of antibiotics, bismuth, or PPI within the prior 4 weeks; patients with allergic history to the medications used; patients with previous gastric surgery; the coexistence of serious concomitant illness (e.g., decompensated liver cirrhosis, uremia); and pregnant women	The infection status of *H. pylori* infection was considered positive if the results met the following criteria (clinical gold standard): positive culture, positive UBT, and concordant positive results in both histology and rapid urease test (RUT). If the patients presented only RUT positive or histology positive, we regarded them as indistinct cases	E-test	Combined detection of urease A gene and Cag A gene for *H. pylori* infection; A2142G and A2143G mutants of the *H. pylori* 23S rRNA gene for CRT	None
Westblom et al. 1993[15]	34	Mean: 52 years Range: from 28 to 76 years	Dyspeptic patients referred for upper gastrointestinal endoscopy and biopsy	Infection with *H. pylori* was defined as a positive culture or the histologic documentation of organisms with characteristic morphology	NA	urease A gene for *H. pylori* infection	None
Kawamula et al. 2003[16]	49	Mean ± SD: 65.1 ± 13.8 years Range: 24-85 years	Patients of early gastric cancer ($n = 25$), gastric ulcer ($n = 9$), and chronic active gastritis ($n = 15$)	*H. pylori* infection was regarded as positive when detected by either rapid urease test (RUT), histological examination, or culture	NA	Combined detection of Cag PAI (i.e., Cag 1, Cag 5, Cag T, Cag E, and cag A) genes and urease B gene for *H. pylori* infection	None
Rimbara et al. 2009[17]	50	Mean ± SD: 54.4 ± 15.2 years	Patients of *H. pylori* infection with or without previous treatment of *H. pylori* eradication	NA	Agar dilution method	A2142G and A2143G mutants of the *H. pylori* 23S rRNA gene for CRT	None

H. pylori, *Helicobacter pylori*; RUT, rapid urease test; ART, antibiotic resistance testing; CRT, clarithromycin resistance testing; NA, not available; E-test, epsilometer tests; SD, standard deviation.

plots shown in Figure 5. The pooled PLR, NLR, DOR, and AUC were 8.79 (95% CI, 4.58-16.86), 0.09 (95% CI, 0.05-0.17), 97.23 (95% CI, 43.74-216.12), and 0.99 (95% CI, 0.98-0.99), respectively.

HSROC curve for diagnosis of clarithromycin resistance is seen approaching the top left-hand corner of the graph with a relatively small prediction region, indicating high accuracy with possibly mild-moderate level of between-study heterogeneity. However, the 95% confidence region around the summary point of sensitivity and specificity was also relatively large, denoting lack of precision (Figure 6).

Figure 5 showed heterogeneity among studies as well, in particular for sensitivity. However, meta-regression

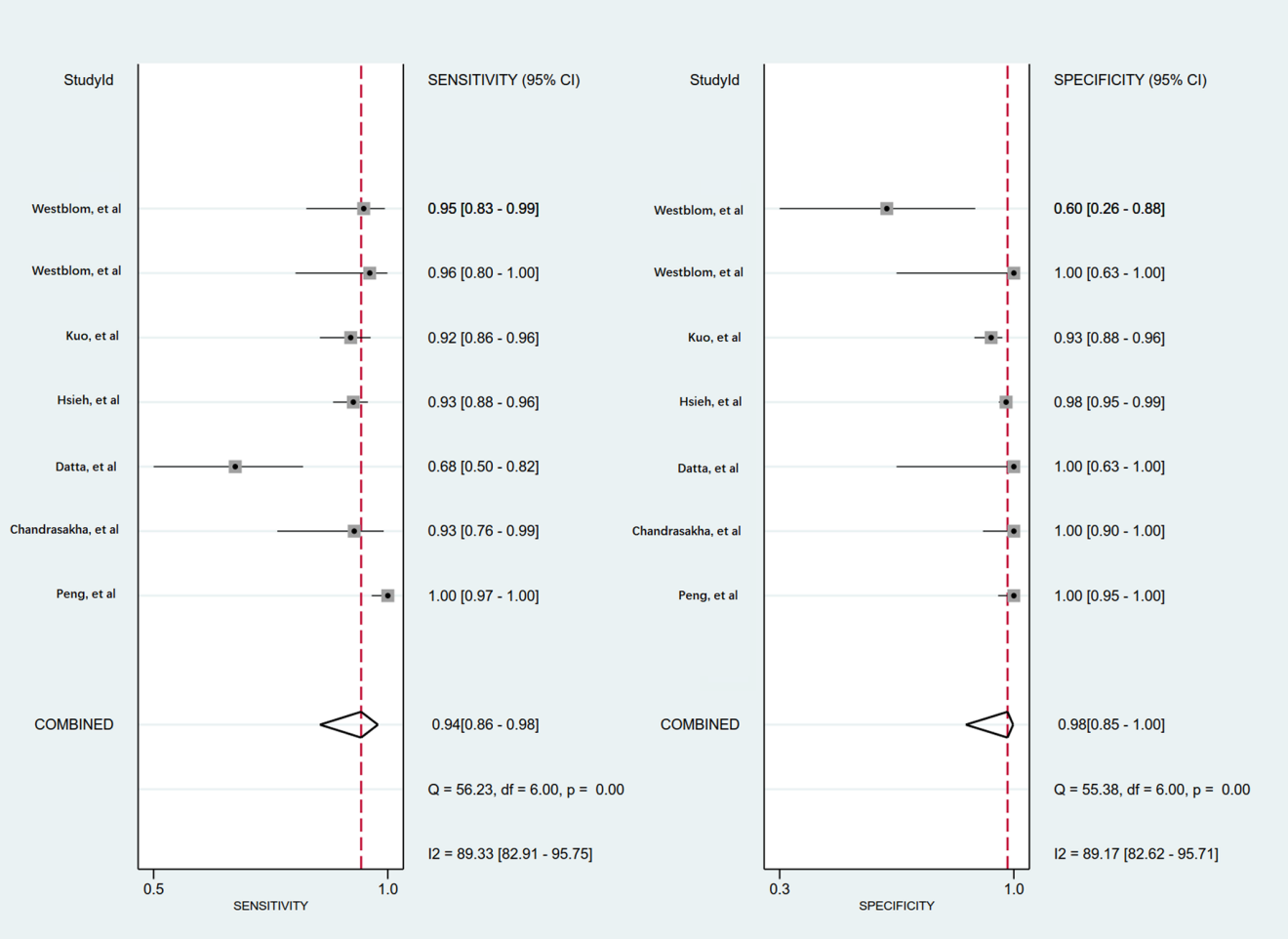

Figure 2. Overall sensitivity and specificity of gastric juice–based genotypic detection of *H. pylori* infection.

analysis was not performed because too few studies ($n = 4$) were included.

Predictive Values

For *H. pylori* infection, with a pretest probability of 47.85%, that is, diagnosis of *H. pylori* infection was 567 of the total included participants ($n = 1185$),[10-16] Fagan plot analysis showed the positive and negative post-test probability of 98% and 5%, respectively (Figure 7A).

For clarithromycin resistance, Fagan plot analysis showed that positive post-test probability was 89% and negative post-test probability was 7%, with pretest probability of 47.09%, that is, clarithromycin resistance was confirmed in 154 of 327 participants in total[10,13,14,17] (Figure 7B).

DISCUSSION

The eradication rate of *H. pylori* has shown a downward trend in recent years.[4] It is wildly accepted that antibiotic resistance may be one of the crucial reasons for the poor eradication rate worldwide. As per the report of World Health Organization (WHO) in 2018, a meta-analysis of antibiotic resistance rate revealed that primary and secondary resistance rates to clarithromycin, metronidazole, and levofloxacin were at an alarming level of ≥15% in all WHO regions. In addition, increasing antibiotic resistance was observed in most WHO regions.[4] It was reported that *H. pylori* may acquire resistance during antibiotic treatment, which made it more difficult to eradicate.[22] Accordingly, many studies explored antibiotic resistance testing and susceptibility-guided therapies (SGT) to improve the eradication rate of *H. pylori*. In 2016, Maastricht V/Florence Consensus of *H. pylori* infection

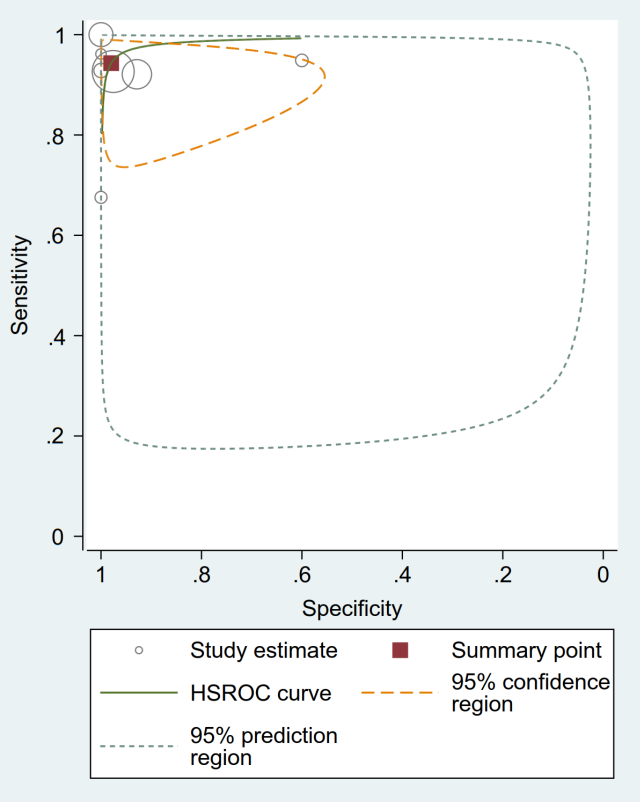

Figure 3. Hierarchical summary receiver-operating characteristic (HSROC) plot of sensitivity and specificity for gastric juice–based genotypic detection of *H. pylori* infection.

management recommended conducting clarithromycin susceptibility test before the standard clarithromycin-based treatment on initial-treated *H. pylori*–infected people especially in the high clarithromycin resistance (<15%) area.[22] However, the indication of antibiotic resistance testing was not clear, especially for patients with previous history of failed eradication therapy. The authors of a recent study hold that occurrence of antibiotic resistance was ascribed to both primary and secondary resistance and specifically associated with failure of clarithromycin-containing regimens.[4] SGTs based on antibiotic resistance testing might increase the chances of eradication and further decrease the frequency of resistance to antibiotics. However, the efficacy of the indication of antibiotic resistance testing was questioned by others. In 2015, López-Góngora et al.[23] performed a meta-analysis of comparison between SGT and empirical antibiotic treatment for *H. pylori* infection. As a result, SGT was found to be superior to empirical 7- or 10-day triple therapy as first-line treatment, but not as second-line treatment. Such results, although of limited evidence quality, questioned the necessity of antibiotic resistance testing prior to administration of second-line therapy. High antibiotic resistance rate and inefficacy of first-line treatment were speculated to be the reasons behind patients to move to second-line therapy.[24] Another limitation of antibiotic resistance testing and SGT was economic implications. Antibiotic resistance testing, especially for culture-based phenotypic methods, is expensive and time consuming.[1] Cost-benefit ratio should be taken into consideration along with improvement of eradication rate.

H. pylori culture–based phenotypic methods and biopsy-based genotypic methods were both currently optional diagnostic tests to detect *H. pylori* with the ability to perform antibiotic resistance testing. However, several

Table 2. Subgroup Analysis and Meta-regression of Gastric Juice–Based Genotypic Detection of *H. pylori* Infection

Subgroup	Number	Sensitivity	*P* Value for Sensitivity	Specificity	*P* Value for Specificity
Participant numbers					
>100	993	96% (92%, 100%)		99% (95%, 100%)	
<100	192	95% (86%, 100%)		92% (73%, 100%)	
>100 vs. <100			.39		**<.01**
Publication type					
Full text	853	94% (88%, 100%)		99% (95%, 100%)	
Non-full text	332	95% (86%, 100%)		92% (73%, 100%)	
Full text vs. Non-full text			.80		**.01**
Study year					
>2010	1057	96% (91%, 100%)		99% (96%, 100%)	
<2010	128	90% (77%, 100%)		92% (73%, 100%)	
>2010 vs. <2010			.41		**<.01**

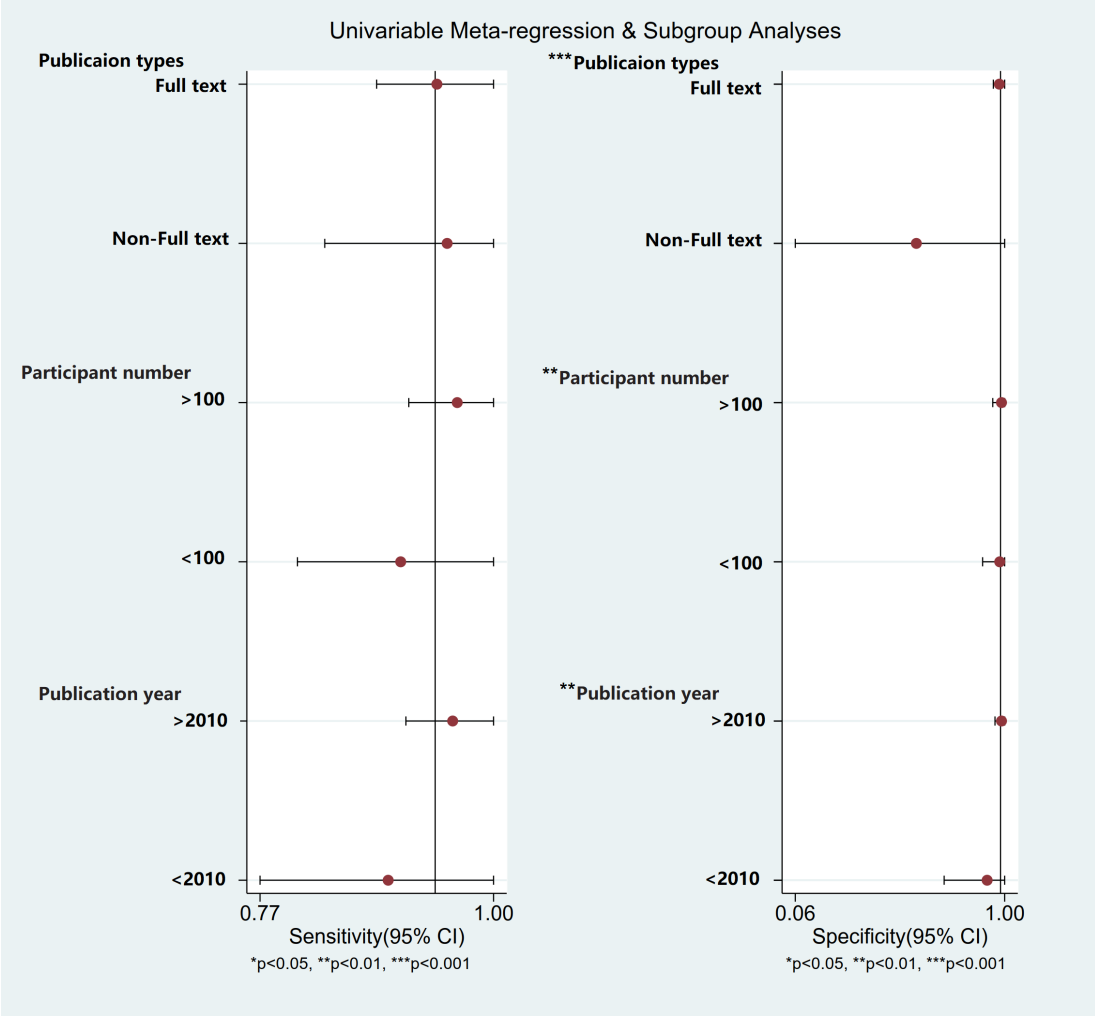

Figure 4. Meta-regression and subgroup analysis for sources of heterogeneity in diagnosis of *H. pylori* infection.

shortcomings limited clinical use of them both. *H. pylori* culture, with certain technical requirements, was observed to show relatively low sensitivity, even as low as 45% in some clinical settings[25] for detecting *H. pylori*. There were many variables such as slow growth rate, low threshold of organisms in clinical specimens, influence on prior therapy, and production of spores,[26] and *H. pylori* culture thus was not recommended for routine diagnosis of *H. pylori* infection.[1] Hence, the antibiotic resistance testing based on *H. pylori* culture was not easily achieved due to a low culture rate. In addition, the methodological shortcomings of being expensive and time consuming made it a non-ideal tool for *H. pylori* management. Compared with *H. pylori* culture, genotypic methods is a highly sensitive technique with lower cost and shorter duration. It is able to diagnose the infection even with few bacteria.[27,28] In 2005, Lo et al.[29] assessed the diagnostic accuracy of PCR assay for *H. pylori* in patients with bleeding peptic ulcers, with results of 96% and 100% sensitivity and specificity, respectively. In 2018, another meta-analysis by Wang et al.[6] evaluated the diagnostic accuracy of PCR-based methods to detect *H. pylori* antibiotic susceptibility in biopsy specimens, with reference standards of phenotypic detection methods. As a result, the pooled sensitivity and specificity of clarithromycin resistance were 96% and 96%, while sensitivity and specificity for quinolone were 97% and 99%. Such results show evidence that biopsy-based genotypic methods might be reliable in the detection of *H. pylori* infection as well as antibiotic resistance. However, several factors challenged this conclusion. First, the uneven distribution of *H. pylori* and random biopsy on

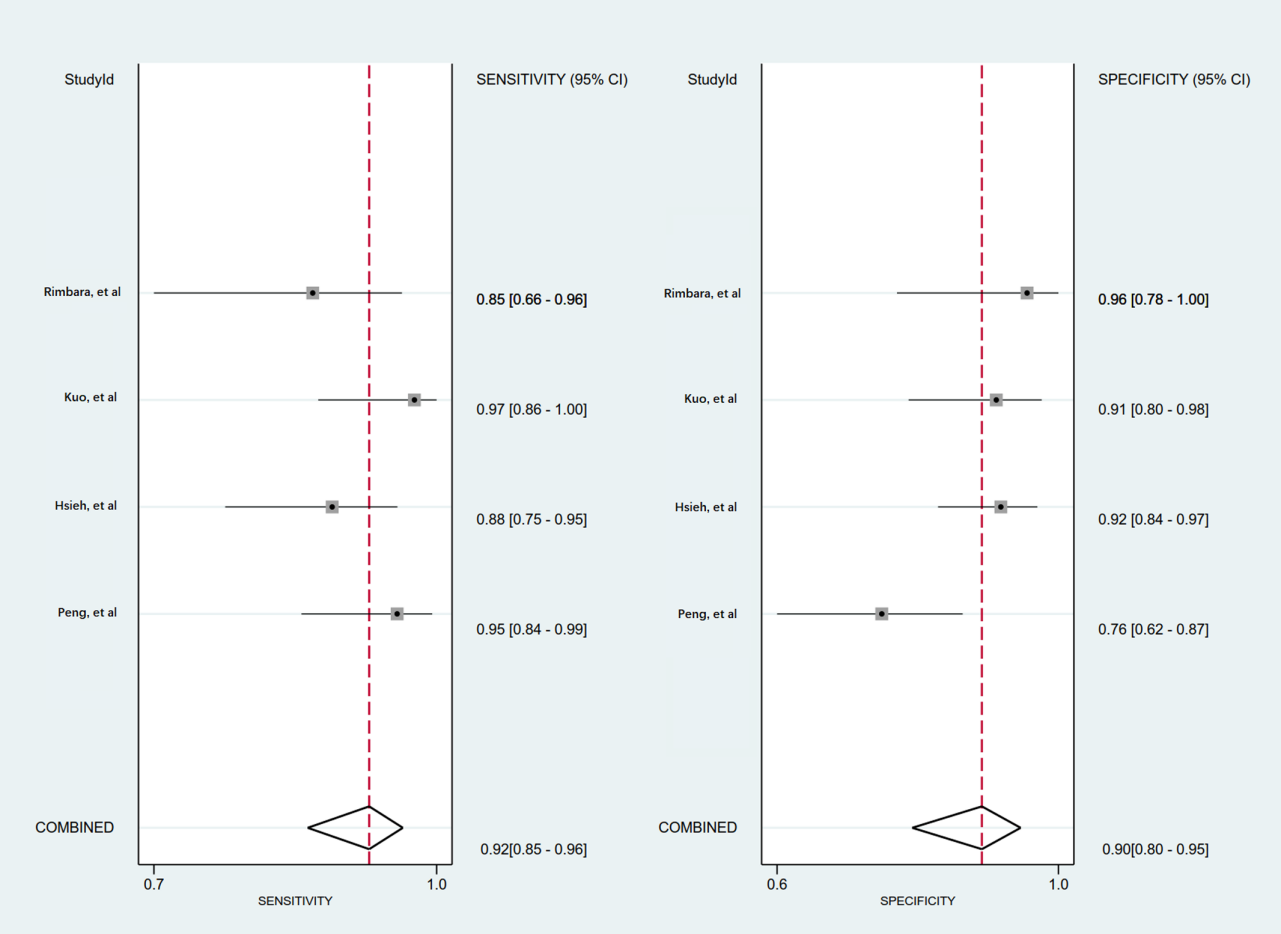

Figure 5. Overall sensitivity and specificity of gastric juice–based genotypic detection of *H. pylori* antibiotic resistance testing.

gastric mucosa might trigger false-negative results due to low-level colonization or absence in certain gastric niches.[28,30] Accordingly, multi-site biopsy was adopted to improve the diagnostic accuracy of *H. pylori* infection,[27,28] which increased the damage due to biopsy. Second, the diagnostic accuracy of biopsy-based genotypic methods was evaluated in the participants with *H. pylori* successfully cultured according to the adopted reference standard.[6] In other words, the *H. pylori* infectors who failed in *H. pylori* culture were not included. Third, some factors such as upper gastrointestinal tract bleeding and peptic ulcers in active stage might lower the density of *H. pylori* and alter its intra-gastric distribution, with increased risk of false-negative results. According to Lo's study,[29] the sensitivity of biopsy-based PCR detection for *H. pylori* infection was 100% in patients without intragastric blood, while it was only 79% in patients with intragastric blood. The decreased intragastric bacterial load during a bleeding ulcer may be a major cause of the reduced sensitivity.[29]

A series of studies have shown that *H. pylori* strains can dissociate from gastric mucosa to gastric juice and can be detected.[13] Such a procedure was not affected by uneven intra-gastric distribution of *H. pylori*.[13] Further, gastric juice–based assay is relatively noninvasive and does not require biopsy, which ensures better safety to patients. What's more, gastric juice–based genotypic methods might function as a comparable diagnostic test of both *H. pylori* detection and antibiotic resistance.

To the best of our knowledge, our study was the first systemic review that includes a meta-analysis of gastric juice–based assay on *H. pylori* detection and antibiotic resistance testing. As a result, the sensitivity

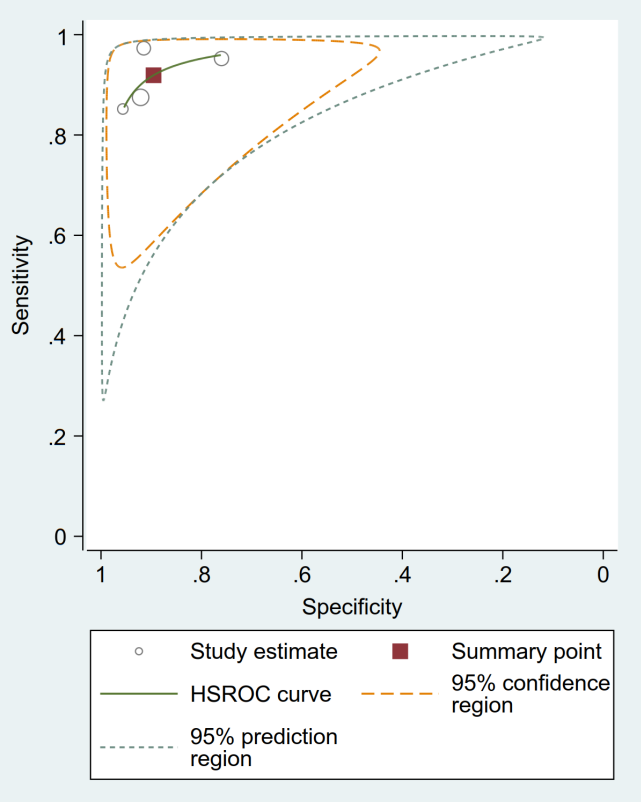

Figure 6. Hierarchical summary receiver-operating characteristic (HSROC) plot of sensitivity and specificity for gastric juice–based genotypic detection of *H. pylori* clarithromycin resistance testing.

and specificity of *H. pylori* detection were 92% and 98%, while those of clarithromycin resistance were 92% and 90%, with a reference standard of phenotypic methods. According to Wang's systematic review, the pooled sensitivity and specificity of biopsy-based genotypic detection methods for detecting clarithromycin resistance were 96% (95% CI: 90%-99%) and 96% (95% CI: 91%-99%), which were prior to our findings of gastric juice–based assay. The possible reasons are as follows. First, the volume of gastric juice in the gastric cavity, especially during endoscopy, differed among populations. The gastric juice might be diluted, which lowered the levels of *H. pylori* in gastric juice. Second, the factors that affect detachment of *H. pylori* from gastric mucosa to gastric juice were unclear. We speculated the amount of *H. pylori* in gastric juice to be closely related to *H. pylori* infection status in gastric mucosa and the entire gastric environment. Further exploration on such influential factors is needed. In addition, both meta-analyses from Wang et al. and us were performed with a reference standard of *H. pylori* culture–based phenotypic methods. The diagnostic accuracy of gastric juice–based genotypic methods in patients with failed *H. pylori* culture was not evaluated. Accordingly, whether gastric juice–based methods were prior to histology-based methods or even prior to *H. pylori* culture–based methods, in such a population was still unclear, which needed further exploration as well.

Several factors contributed to the heterogeneity. On one hand, the criteria of patient selection in each study were different. At the same time, only two studies[10,11] stated that a consecutive or random sample of patients was enrolled. Only one study[14] enrolled indistinct cases, while others included *H. pylori*–infected patients and non-*H. pylori*–infected ones, but none of unclear diagnosis, as per the reference standard. Many factors made it difficult to detect *H. pylori*, especially decreased bacterial quantity in gastric mucosa or deep-seated colonization in gastric mucosa. Reliability of gastric juice–based genotypic methods in such patients was still unclear. On the other hand, we included the studies with reference standards of combination of at least two diagnostic tests, that is urea breath test, rapid urease test, histology, and *H. pylori* culture. However, the adoption of such diagnostic tests differed among the included studies. What's more, the target genes varied as well, including Urea genes, Gag genes, and combined detection of genes. Such differences in reference standards of *H. pylori* might contribute to clinical heterogeneity.

There were some limitations in the present review. First, both methodological bias and clinical bias existed, as mentioned earlier. Second, the number of studies included was relatively few. Only four studies explored the diagnostic efficacy of gastric juice–based antibiotic resistance testing. Further studies on this issue are still needed. In addition, only clarithromycin resistance testing has been referred to. The studies do not highlight information on quinolone and metronidazole, while the latter two were also widely used with erstwhile reporting of high resistance rate.

In conclusion, gastric juice–based genotypic methods are reliable for diagnoses of *H. pylori* infection as well as clarithromycin resistance. However, such results should be treated with caution due to limited evidence.

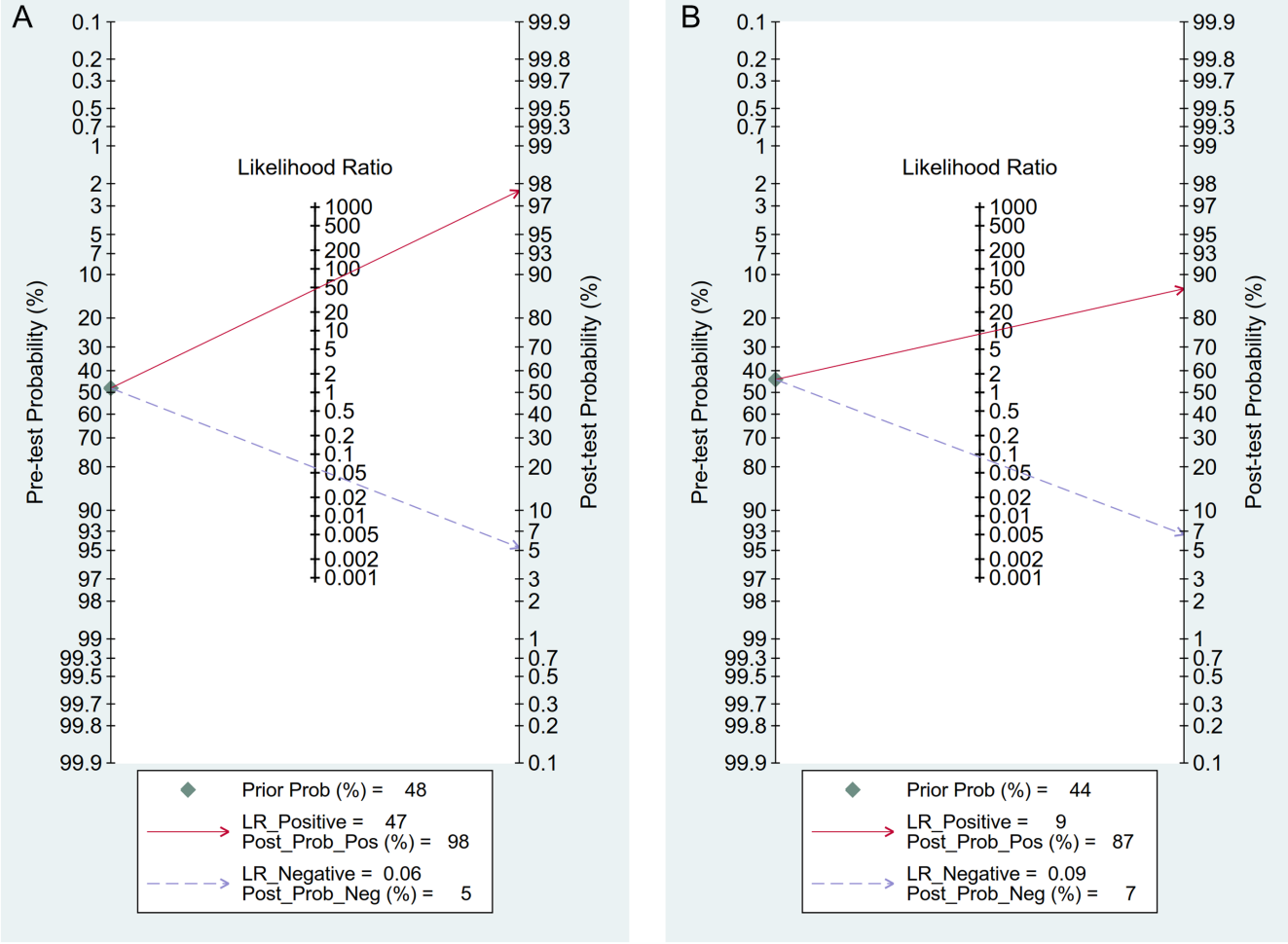

Figure 7. (A) Fagan's plot for the post-test probability of *H. pylori* infection after a positive result (upper line) or negative result (lower line) of gastric juice–based genophetic detection. (B) Fagan's plot for the post-test probability of clarithromycin resistance after a positive result (upper line) or negative result (lower line) of gastric juice–based genophetic detection.

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