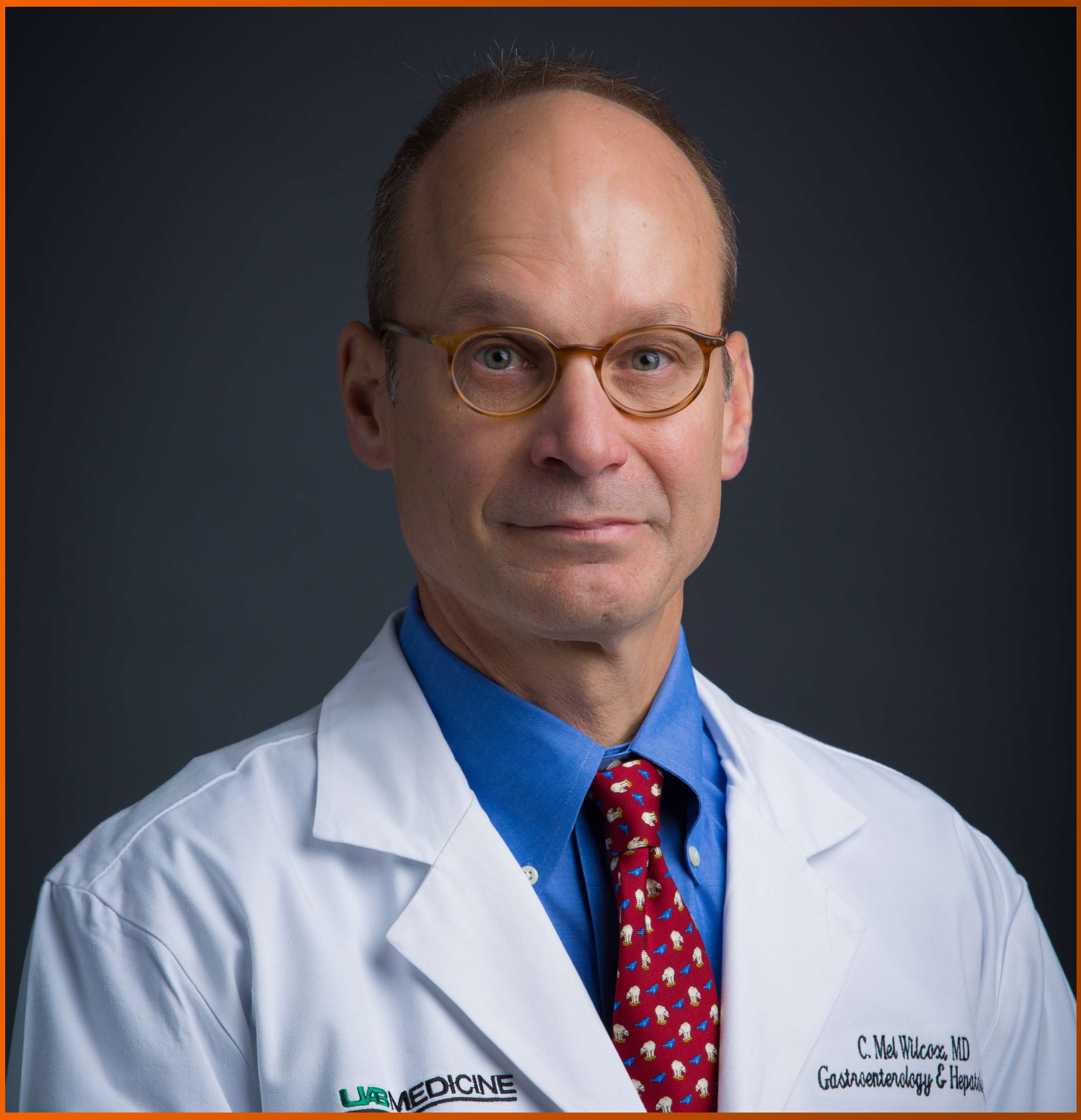
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

World Journal of *Gastroenterology*

World J Gastroenterol 2018 July 21; 24(27): 2921-3054



Published by Baishideng Publishing Group Inc

World Journal of Gastroenterology

Contents

Weekly Volume 24 Number 27 July 21, 2018

ABOUT COVER

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AIMS AND SCOPE

World Journal of Gastroenterology (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a peer-reviewed open access journal. *WJG* was established on October 1, 1995. It is published weekly on the 7th, 14th, 21st, and 28th each month. The *WJG* Editorial Board consists of 642 experts in gastroenterology and hepatology from 59 countries.

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NAME OF JOURNAL
World Journal of Gastroenterology

ISSN
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

LAUNCH DATE
October 1, 1995

FREQUENCY
Weekly

EDITORIAL OFFICE
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PUBLICATION DATE
July 21, 2018

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World Journal of Gastroenterology

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World J Gastroenterol 2018 July 21; 24(27): 3021-3029

DOI: 10.3748/wjg.v24.i27.3021

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Clinical Trials Study

New fecal test for non-invasive *Helicobacter pylori* detection: A diagnostic accuracy study

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Author contributions: Iannone A, Di Leo A and Ierardi E designed the research; Iannone A, Giorgio F, Russo F, Riezzo G, Girardi B, Pricci M and Principi M performed the research; Giorgio F, Russo F, Riezzo G, Girardi B and Pricci M collected the data; Iannone A analyzed the data; Iannone A, Palmer SC, Strippoli GF and Ierardi E wrote the paper; Palmer SC, Barone M, Principi M, Strippoli GF, Di Leo A and Ierardi E critically revised the manuscript for important intellectual content.

Institutional review board statement: The study was performed in agreement with the ethical guidelines of the Declaration of Helsinki and the protocol was approved by the local Ethics Committee (Ospedale Consorziale Policlinico, Bari, protocol number 74413).

Informed consent statement: All participants gave written informed consent before inclusion in the study.

Conflict-of-interest statement: Alfredo Di Leo is an advisory board member of THD Spa. Floriana Giorgio, Bruna Girardi and Maria Pricci are employees of THD Spa. All other authors declare no financial support or conflict of interest.

Data sharing statement: The raw data and SAS code used for all analyses are available from the corresponding author.

Manuscript source: Invited manuscript

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Received: April 28, 2018
Peer-review started: April 28, 2018

First decision: June 11, 2018
Revised: June 12, 2018
Accepted: June 27, 2018
Article in press: June 27, 2018
Published online: July 21, 2018

Abstract

AIM

To assess the diagnostic accuracy of a new fecal test for detecting *Helicobacter pylori* (*H. pylori*), using ^{13}C-urea breath test as the reference standard, and explore bacterial antibiotic resistance.

METHODS

We conducted a prospective two-center diagnostic test accuracy study. We enrolled consecutive people ≥ 18 years without previous diagnosis of *H. pylori* infection, referred for dyspepsia between February and October 2017. At enrollment, all participants underwent ^{13}C-urea breath test. Participants aged over 50 years were scheduled to undergo upper endoscopy with histology. Participants collected stool samples 1-3 d after enrollment for a new fecal investigation (THD fecal test). The detection of bacterial 23S rRNA subunit gene indicated *H. pylori* infection. We also used the index diagnostic test to examine mutations conferring resistance to clarithromycin and levofloxacin. Independent investigators analyzed index test and reference test standard results blinded to the other test findings. We estimated sensitivity, specificity, positive (PPV) and negative (NPV) predictive value, diagnostic accuracy, positive and negative likelihood ratio (LR), together with 95% confidence intervals (CI).

RESULTS

We enrolled 294 consecutive participants (age: Median 37.0 years, IQR: 29.0-46.0 years; men: 39.8%). Ninety-five (32.3%) participants had a positive ^{13}C-urea breath test. Twenty-three (7.8%) participants underwent upper endoscopy with histology, with a full concordance between ^{13}C-urea breath test and histology in detecting *H. pylori* infection. Four (1.4%) out of the 294 participants withdrew from the study after the enrollment visit and did not undergo THD fecal testing. In the 290 participants who completed the study, the THD fecal test sensitivity was 90.2% (CI: 84.2%-96.3%), specificity 98.5% (CI:96.8%-100%), PPV 96.5% (CI: 92.6%-100%), NPV 95.6% (CI: 92.8%-98.4%), accuracy 95.9% (CI: 93.6%-98.2%), positive LR 59.5(CI: 19.3-183.4), negative LR 0.10 (CI: 0.05-0.18). Out of 83 infected participants identified with the THD fecal test, 34 (41.0%) had bacterial genotypic changes consistent with antibiotic-resistant *H. pylori* infection. Of these, 27 (32.5%) had bacterial strains resistant to clarithromycin, 3 (3.6%) to levofloxacin, and 4 (4.8%) to both antibiotics.

CONCLUSION

The THD fecal test has high performance for the non-invasive diagnosis of *H. pylori* infection while additionally enabling the assessment of bacterial antibiotic resistances.

Key words: *Helicobacter pylori*; Fecal test; Feces; Stools; 23S rRNA; Molecular analysis; Antibiotic resistance; Diagnostic accuracy

Core tip: Existing studies on molecular tests for *Helicobacter pylori* (*H. pylori*) detection in stools show suboptimal quality. The THD fecal test is a newer method to detect bacterial DNA and mutations conferring antibiotic resistance. In this diagnostic test accuracy study involving unselected consecutive participants and blinded outcome assessment, we evaluated the diagnostic accuracy of the THD fecal test for detecting *H. pylori*, using the ^{13}C-urea breath test as the reference standard. We found that the THD fecal test has high performance for the non-invasive diagnosis of *H. pylori* infection while additionally enabling the assessment of bacterial antibiotic resistances.

Iannone A, Giorgio F, Russo F, Riezzo G, Girardi B, Pricci M, Palmer SC, Barone M, Principi M, Strippoli GF, Di Leo A, Ierardi E. New fecal test for non-invasive *Helicobacter pylori* detection: A diagnostic accuracy study. *World J Gastroenterol* 2018; 24(27): 3021-3029 Available from: URL: http://www.wjgnet.com/1007-9327/full/v24/i27/3021.htm DOI: http://dx.doi.org/10.3748/wjg.v24.i27.3021

INTRODUCTION

Helicobacter pylori (*H. pylori*) infection occurs among 48.5% of the general population worldwide, with high geographic variability[1]. It is the leading cause of chronic/atrophic gastritis, peptic ulcer, gastric lymphoma, gastric carcinoma, and some extra-gastric disorders[2-4]. Diagnostic approaches for *H. pylori* infection include invasive and non-invasive testing[4-6]. The ^{13}C-urea breath test is established as a highly sensitive and specific test (96% sensitivity and 93% specificity) for the non-invasive diagnosis ofinfection[4,7]. A stool-based monoclonal antigen test has low acceptability in some contexts and needs local validation, despite high accuracy[4,8]. Invasive tests require upper endoscopy, limiting their application based on local practices and policies and to patients who have alarm symptoms[4,5,9]. Culture with antibiogram is only recommended after repeated treatment failures[4-6] due to its high false negative rate[10,11].

Molecular testing is a promising approach for diagnosing *H. pylori* infection and has the added advantage of identifying bacterial DNA mutations associated with antibiotic resistance. Molecular tests on gastric biopsy are commonly used only for research purposes due to

the need for an invasive endoscopic procedure[12].Thus, the application of these tests on fecal samples is gaining interest. A recent meta-analysis identified the bacterial 23S ribosomal RNA subunit gene as the most accurate marker for diagnosis of infection using molecular tests on stool samples, with 82% (95%CI: 77%-86%) sensitivity and 99%(95%CI: 98%-100%) specificity[13]. The consistency of these results is limited by the inclusion of studies of suboptimal quality resulting from bias in participant selection and lack of blinded outcome assessment[14-19].

We aimed to assess the diagnostic accuracy of a new molecular test, theTHD fecal test[20], for the non-invasive detection of *H. pylori* DNA, using the ^{13}C-urea breath test as the reference standard. We estimated the point prevalence of *H. pylori* DNA point mutations conferring resistance to clarithromycin and levofloxacin.

MATERIALS AND METHODS

This study was conducted according to the Standards for Reporting of Diagnostic Accuracy (STARD) statement[21].

Study design

We performed a two-center cross-sectional study with prospective data collection. We included consecutive participantsexperiencing dyspeptic symptoms and without previous diagnosis of *H. pylori* infection, who were referred for diagnostic evaluation to the Gastroenterology Unit, University of Bari (Italy) or the National Institute of Gastroenterology "Saverio De Bellis", CastellanaGrotte, Bari (Italy) between February and October 2017.

Participants were eligible if they were aged 18 years or older and had experienced dyspeptic symptoms, defined as the presence of one or more of: post-prandial fullness, early satiation, epigastric pain and epigastric burning for at least one (post-prandial fullness and early satiation) or three (epigastric pain and epigastric burning) days per week in the last three months with symptoms onset at least six months previously[22]. Exclusion criteria were treatment with proton pump inhibitors or 2-histamine receptor antagonists in the previous two weeks as well as use of antibiotics or bismuth salts in the previous four weeks, as these medications may increase false negative results of invasive and non-invasive current diagnostic tests for *H. pylori* infection by reducing the bacterial load[23-25]. Additional exclusion criteria were previous diagnosis of *H. pylori* infection and presence of chronic diarrhea, which can limit the accurate collection of stool samples for the THD fecal test. Potential participants were also excluded if they had alarm symptoms, including weight loss, dysphagia, gastrointestinal bleeding, an abdominal mass or iron deficiency anemia, which are an indication to perform upper endoscopy as a first-line diagnostic approach[4,5,9].

At the enrollment visit, eligible participants underwent the ^{13}C-urea breath test (the reference standard) for the non-invasive investigation of *H. pylori* infection. According to current Italian Clinical guideline recommendations[5], participants older than 50 years were scheduled to undergo upper endoscopy with biopsy sampling for histology within one week. All participants were asked to provide stool samples collected 1 to 3 d after enrollment, using the THD device. These samples were used for the THD fecal test (index test). Independent investigators analyzed the index test and reference standard test results blinded to the other test findings, participants' information and histology results. Pathologists performing histology examination were unaware of the results of the other two tests.

Thestudy was performed in agreement with the ethical guidelines of the Declaration of Helsinki and the protocol was approved by the local Ethics Committee (Ospedale Consorziale Policlinico, Bari, protocol number 74413). All participants gave written informed consent before inclusion in the study.

THD fecal test (index test)

The technical details of *H. pylori* DNA extraction and analysis are reported in Appendix 1.

Within three days after the enrollment visit, participants collected and stored a stool sample using the THD fecal test equipment (THD Spa, Correggio, Reggio Emilia, Italy), which allows for obtaining an adequate stool-derived product to extract *H. pylori* DNA.

We pre-specified THD fecal test positivity as the identification of the *H. pylori* bacterial gene encoding the 23S ribosomal RNA subunit in the stool-derived product[20]. The detection of specific bacterial DNA point mutations indicated *H. pylori* resistance to clarithromycin and/or levofloxacin. In brief, we assessed A2142C, A2142G and A2143G point mutations in the 23S rRNA subunit gene for clarithromycin resistance, and C261A, C261G, G271A, A272G, G271T and A270T point mutations in the A-subunit of gyrase gene for levofloxacin resistance.

^{13}C-urea breath test (reference standard) and upper endoscopy

The technical details of the ^{13}C-urea breath test are reported in Appendix 2.

At the enrollment visit, all participants underwent ^{13}C-urea breath testing after overnight fasting. We used this test as the reference standard due to the non-invasiveness, high diagnostic accuracy (sensitivity of 96% and specificity of 93%), and consumer acceptance[4,7]. We pre-specified a ^{13}C-urea breath test positivity for a difference between the baseline and 30 min breath sample that exceeded 4 parts per 1000 of ^{13}carbon dioxide ($^{13}CO_2$)[26,27].

Participants older than 50 years were scheduled to undergo upper endoscopy with biopsy sampling for histology, according to current Italian guidelines[5]. Among these participants, a minimum of two biopsy

Table 1 Characteristics of the participants included in the study *n* (%)

Characteristic	Value (*n* = 294)
Age, median (IQR), yr	37.0 (29.0-46.0)
Female sex, number	177 (60.2)
Helicobacter pylori infection, number	95 (32.3)[1]
Upper endoscopy with histology, number	23 (7.8)[2]
Dyspeptic symptoms, number	
Post-prandial fullness	115 (39.1)
Early satiation	50 (17.0)
Epigastric pain	130 (44.2)
Epigastric burning	170 (57.8)
Concomitant diseases, number	
Cardiovascular disease	8 (2.7)
Chronic kidney disease/dialysis	0 (0)
Chronic liver disease/cirrhosis	3 (1.0)
Other chronic diseases[3]	10 (3.4)

[1]Diagnosed with the ^{13}C-urea breath test (reference standard test); [2]There were 40 participants older than 50 years, but 17 refused to undergo upper endoscopy; [3]Including asthma (8 participants), spasmophilia (1 participant), multiple sclerosis (1 participant). IQR: Interquartile range.

samples from the gastric antrum (greater and lesser curvature, 3 cm proximal to the pyloric region) and two from the middle of the gastric body were collected for histologic examination[4].

Statistical analysis

We assessed the normal distribution of continuous variables using the Shapiro-Wilk test and expressed them as the mean and standard deviation (SD) or median and interquartile range (IQR). We expressed categorical variables asa percentage.

The results of the ^{13}C-urea breath test served as the reference standard for assessing the diagnostic accuracy of the THD fecal test in detecting H. *pylori* infection. We calculated sensitivity, specificity, negative and positive predictive values, and diagnostic accuracy for the THD fecal test together with 95% confidence intervals (CI), according to standard definitions. Since the prevalence of the condition in the enrolled population influences sensitivity, specificity, and predictive values, we also calculated the positive and negative likelihood ratios. To identify the impact of our findings on clinical decision-making, we calculated the post-test probability after positive and negative results onthe THD fecal test for populations with different pre-test probabilities of *H. pylori* infection based on likelihood ratios.

We pre-planned to handle uninterpretable and missing index test or reference standard findings with both the "complete case" approach and "best-worst case" imputation, to avoid overestimation of diagnostic accuracy parameters[28]. In detail, these results were removed for the "complete case" analysis, while they were considered as false-negatives and false-positivesfor the "worst case" analysis or as true-negatives and true-positives for the "best-case" analysis.

We estimatedthe point prevalence of *H. pylori* resistance to clarithromycin and levofloxacinas the number of participant with the specific antibiotic resistance divided by the total number of participants diagnosed with *H. pylori* infection at THD fecal test.

We assumed a 94% sensitivity and 97% specificity for the THD fecal test, based on monoclonal stool antigen test accuracy parameters for the lack of high-quality evidence on molecular fecal tests[8]. Assuming a *H. pylori* infection prevalence of 34.3%[1], a marginal error of 0.05, and a drop-out of 10%, we calculated a sample size of at least 280 participants, to provide 80% power with an α of 0.05 to detect the pre-specified sensitivity and specificity values for the THD fecal test[29].

We used Statistical Analysis Software (SAS Institute Inc., Cary, NC, United States) 9.4 for all the analyses.

RESULTS

Characteristics of study population

During the enrollment period, 305 consecutive participants were eligible for inclusion in the study. Of these, 11 were excluded for preference not to participate.Four out of the 294 participants withdrew from the study after the enrollment visit did not undergo THD fecal testing (Figure 1).

Table 1 describes the characteristics of the 294 participants included in the study. The median age was 37.0 years (IQR: 29.0 to 46.0 years). There were 177 (60.2%) women. The ^{13}C-urea breath test (reference standard test) was positive in 95 (32.3%) participants. Forty (13.6%) participants were older than 50 years and were scheduled to undergo upper endoscopy with histology. Of these, 23 (7.8%) participants, 7 with and 16 without *H. pylori* infection, agreed to undergo this examination. There was full concordance between the ^{13}C-urea breath test and histology in detecting *H. pylori* infection in these participants. With regard to dyspeptic symptoms reported at enrollment visit, 115 (39.1%) participants experienced post-prandial fullness, 50 (17.0%) early satiation, 130 (44.2%) epigastric pain, and 170 (57.8%) epigastric burning. There were no reported adverse events during performance of the index or reference standard test.

THD fecal test diagnostic performance

The amplification curves of *H. pylori* DNA sequences from the real time polymerase chain reaction are shown in Appendix 3.

Direct comparisons between the ^{13}C-urea breath test and THD fecal test results for detecting *H. pylori* infection are reported in Table 2.

The diagnostic accuracy parameters of the THD fecal test for detecting *H. pylori* infection, using ^{13}C-urea breath test as the reference standard, are shown in Table 3. In the "complete-case" analysis, including the 290 participants who completed the study, the THD

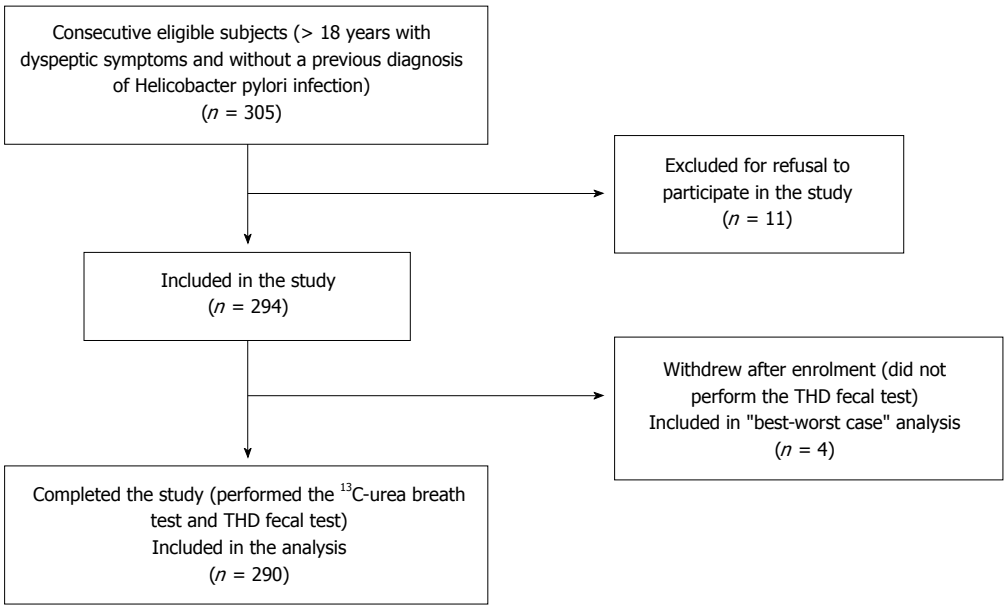

Figure 1 Flow diagram of participant recruitment in the study.

fecal test had a sensitivity of 90.2% (CI: 84.2% to 96.3%), specificity of 98.5% (CI: 96.8% to 100%), positive predictive value of 96.5% (CI: 92.6% to 100%), and negative predictive value of 95.6% (CI: 92.8% to 98.4%). The accuracy of the index test in correctly identifying participants with and without *H. pylori* infection was 95.9% (CI: 93.6% to 98.2%). The THD fecal test positive likelihood ratio was 59.5 (CI: 19.3 to 183.4), and the negative likelihood ratio was 0.10 (CI: 0.05 to 0.18). In the "best-worst case" analysis, including all 294 participants enrolled in the study, there were small changes in THD fecal test diagnostic accuracy parameters (Table 3).

Figure 2 displays the estimation of the probability of *H. pylori* infection after positive and negative THD fecal test results for populations with different prevalence of disease, based on likelihood ratios calculated from our data. As shown, the probability of infection after a positive index test finding is higher than 90% in populations with a disease prevalence ≥ 20%. The probability of infection after a negative index test finding is lower than 10% and 20% in populations with disease prevalence ≤ 50% and ≤ 70%, respectively.

H. pylori resistance rates to clarithromycin and levofloxacin

Table 4 summarizes the *H. pylori* resistance rates to clarithromycin and levofloxacin in the 83 *H. pylori* infected participants identified with the THD fecal test. Thirty-four (41.0%) participants had bacterial genotypic changes consistent with antibiotic-resistant *H. pylori* infection. Of these, 27 (32.5%) had bacterial strains resistant to clarithromycin, 3 (3.6%) to levofloxacin, and 4 (4.8%) to both antibiotics. The overall resistance rates were 37.3% (31 participants) to clarithromycin and 8.4% (7 participants) to levofloxacin.

DISCUSSION

Main findings

In this diagnostic test accuracy study involving unselected consecutive participants and blinded outcome assessment, we observed that the non-invasive THD fecal test has high performance for the diagnosis of *H. pylori* infection among patients with dyspeptic symptoms. This test showed a 90.2% sensitivity and 98.5% specificity, with a diagnostic accuracy of 95.9%. Considering the worldwide prevalence of *H. pylori* infection, ranging from 70% in Africa to 24% in Oceania[1], there was > 90% post-test probability of bacterial infection after a positive THD fecal test result and < 20% probability after a negative finding. In Western Europe, the prevalence of infection is around 34%[1], leading to post-test probabilities > 95% and < 5% after a positive and negative test, respectively. The THD fecal test identified *H. pylori* resistance rates of 32.5% to clarithromycin, 3.6% to levofloxacin, and 4.8% to both antibiotics.

Comparison with existing knowledge

A recent meta-analysis found that the 23S rRNA subunit gene is the most accurate marker for detecting *H. pylori* in stools using molecular analyses based on real time polymerase chain reaction. The pooled analysis of six diagnostic accuracy studies showed estimated sensitivity of 82% (95%CI: 77% to 86%) and specificity of 99% (95%CI: 98% to 100%) for the bacterial 23S rRNA subunit gene[13]. Comparing our results with findings from existing primary studies[14-19] included in this meta-analysis is difficult, mainly due to

Table 2 Direct comparisons between the ^{13}C-urea breath test and THD fecal test results for detecting participants with *Helicobacter pylori* infection

	^{13}C-urea breath test	
	Positive	Negative
THD fecal test		
Positive	83	3
Negative	9	195
Missing[1]	3	1
Total	95	199

[1]Four participants withdrew from the study after enrollment, thus they underwent the ^{13}C-urea breath test but not the THD fecal test.

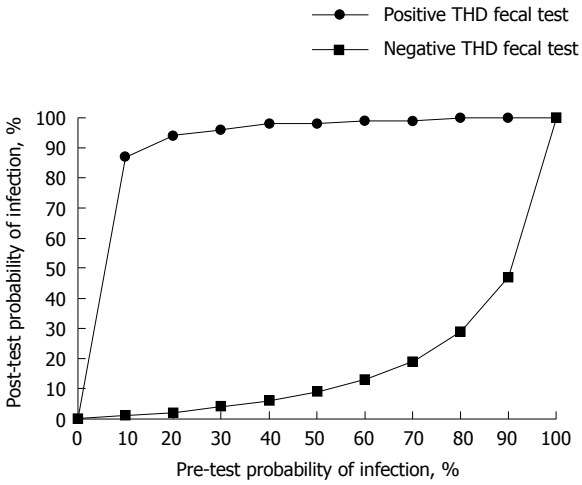

Figure 2 Post-test probability after positive and negative results on the THD fecal test for populations with different pre-test probabilities of *Helicobacter pylori* infection, based on likelihood ratios.

limitations in methodological reporting in these studies and differences in the selection of population and the reference standard used. Four studies[14,15,17,19] included children (age < 18 years), one[18] did not specify the participants' age, and one[16] included adults (range 20 to 81 years), with an estimated disease prevalence ranging between 21% and 81% among studies. The reference standard was monoclonal stool antigen test in three studies[16-18], a combination of histology, rapid urease test, and culture in two studies[14,19], and a combination of histology, culture, ^{13}C-urea breath test and monoclonal stool antigen test in one study[15]. In two studies[14,19], consecutive participants were enrolled, while in the remaining four[15-18], a convenience sample was selected. No authors provided the sample size estimation. There was no reported blinding of the assessment of the index and reference standard test findings, except for pathologists analyzing gastric biopsy samples in one study[15]. Thus, the use of convenience samples and lack of blinding may have led to an overestimation of the diagnostic accuracy of these molecular tests for the non-invasive diagnosis of *H. pylori* infection. Despite the selection of consecutive participants and the blinded assessment of the index and reference standard results in our study, we found greater test sensitivity and similar high specificity compared to the pooled estimate of earlier studies. This may be due to the high performance of the THD device for obtaining adequate stool sample-derived product to extract *H. pylori* DNA. Our sensitivity estimate is concordant with that reported in a recent publication[30], although this last study is burdened by the same methodological limitations and risk of bias as previous researches.

Our *H. pylori* resistance rates to clarithromycin and levofloxacinwere consistent with those reported in previous epidemiologic studies from the same geographic area[31]. Molecular analyses performed on stool samples showed bacterial resistance rates of 36%-41% to clarithromycin[16,30], in agreement with our estimate of 37.3%. There is limited evidence on the use of molecular fecal tests to detect *H. pylori* resistance to levofloxacin. Recently, the molecular analysis "Genotype HelicoDR assay" (Hain Lifescience GmbH, Nehren, Germany) has been applied to fecal samples for detecting clarithromycin and fluoroquinolone resistance using molecular analysis on gastric biopsy, with proven high accuracy, as the reference standard[32]. This test identified a resistance rate to fluoroquinolone of 13% in stools, which was somewhat higher than the present study (8.4%). However, there was low agreement between stool and biopsy findings for resistance to both clarithromycin (53%) and fluoroquinolone (35%), indicating a poor performance of this test for the non-invasive assessment of *H. pylori* resistances to antibiotics. By contrast, the THD fecal test has shown high concordance with real time polymerase chain reaction-based molecular analysis on gastric tissue for detecting bacterial resistance to clarithromycin[20].

Strengths and limitations

This study investigated the diagnostic accuracy parameters of a novel molecular tool (THD fecal test) for the non-invasive diagnosis of *H. pylori* infection in consecutive participants with dyspepsia. The strengths of the study include an *a priori* sample size, prospectively enrolled consecutive participants, and blinded assessment of the index and reference standard results to increase the certainty of the findings. Our study also assessed the feasibility of detecting bacterial resistance to clarithromycin and levofloxacin, using a non-invasive approach.

Our study has some limitations that should be considered when interpreting the results. First, we diagnosed *H. pylori* infection using a single non-invasive test, while a second confirmation test (histology) was performed only in the subgroup of participants older than 50 years. However, the ^{13}C-urea breath test is recommended as the gold-standard non-invasive diagnostic approach for detecting this bacterial infection, with 96% sensitivity and 93% specificity[4,7]. We also

Table 3 Diagnostic accuracy parameters of the THD fecal test for detecting *Helicobacter pylori* infection, using ^{13}C-urea breath test as the reference standard

Parameter	Complete-case analysis (n = 290)	Best-case analysis (n = 294)[1]	Worst-case analysis (n = 294)[1]
Sensitivity, % (95%CI)	90.2 (84.2 to 96.3)	90.5 (84.6 to 96.4)	87.4 (80.7 to 94.1)
Specificity, % (95%CI)	98.5 (96.8 to 100)	98.5 (96.8 to 100)	98.0 (96.0 to 99.9)
PPV, % (95%CI)	96.5 (92.6 to 100)	96.6 (92.9 to 100)	95.4 (91.0 to 99.8)
NPV, % (95%CI)	95.6 (92.8 to 98.4)	95.6 (92.8 to 98.4)	94.2 (91.0 to 97.4)
Accuracy, % (95%CI)	95.9 (93.6 to 98.2)	95.9 (93.7 to 98.2)	94.6 (92.0 to 97.2)
Positive LR, estimate (95%CI)	59.5 (19.3 to 183.4)	60.0 (19.5 to 185.0)	43.5 (16.4 to 115.0)
Negative LR, estimate (95%CI)	0.10 (0.05 to 0.18)	0.10 (0.05 to 0.18)	0.13 (0.08 to 0.22)

[1]The analysis includes four participants with missing data for the THD fecal test. PPV: Positive predictive value; NPV: Negative predictive value; LR: Likelihood ratio; CI: Confidence interval.

Table 4 *Helicobacter pylori* resistance rates to clarithromycin and levofloxacin in the 83 infected participants identified with the THD fecal test n (%)

	Clarithromycin		Total
	Susceptible	Resistant	
Levofloxacin			
Susceptible	49 (59.1)	27 (32.5)	76 (91.6)
Resistant	3 (3.6)	4 (4.8)	7 (8.4)
Total	52 (62.7)	31 (37.3)	83 (100)

found complete agreement between ^{13}C-urea breath test and histology in participants older than 50 years. Moreover, current international guidelines[4,5,9] recommend a test-and-treat strategy for *H. pylori* infection in young (≤ 50 years in Italy) dyspeptic people without alarm symptoms to avoid the costs, inconvenience and discomfort of endoscopy. Thus, our approach reflects the current clinical practice and the most appropriate diagnostic strategy for this population. Second, we did not perform molecular analysis of bacterium resistance to clarithromycin and levofloxacin on gastric biopsy samples to confirm the results obtained on stool samples. Thus, we did not calculate diagnostic accuracy parameters of the THD fecal test for detecting the bacterial resistance to these antibiotics. However, we have previously demonstrated full concordance between THD fecal test and molecular analysis on gastric tissue findings for detecting *H. pylori* resistance to clarithromycin[20].

Implications for practice and research / conclusions

In conclusion, our results indicate that the THD fecal test has high diagnostic performance for non-invasive detection of *H. pylori* infection in patients with dyspeptic symptoms while enabling identification of bacterium resistance to clarithromycin and levofloxacin. The THD fecal test may assist in the conduct of randomized trials to evaluate the benefits and harms of tailored eradication strategies in first-line. On these bases, the THD fecal test may inform clinical decision-making and guide individualized treatments for *H. pylori* infection.

ARTICLE HIGHLIGHTS

Research background

Diagnostic approaches for *Helicobacter pylori* (*H. pylori*) infection include invasive and non-invasive testing. The non-invasive ^{13}C-urea breath test and stool monoclonal antigen test have high accuracy for diagnosing the infection, although the stool test has low acceptability in some contexts and needs local validation. The need for upper endoscopy is a limitation to the use of invasive tests. Molecular tests are promising approaches for diagnosing *H. pylori* infection, due to the added advantage of identifying bacterial DNA mutations associated with antibiotic resistance.

Research motivation

The application of molecular diagnostic tests on gastric biopsy samples is limited by the need for invasive endoscopic procedure. Thus, the non-invasive application of these tests on fecal samples is gaining increasing interest. An accurate non-invasive molecular test may guide first-line eradicating treatments with the potential advantages of increasing bacterial eradication rates and reducing the development of *H. pylori* resistance to antibiotics. However, existing studies on molecular tests for *H. pylori* detection in stools show suboptimal quality.

Research objectives

We aimed to assess the accuracy of a new non-invasive molecular test, the THD fecal test, for the diagnosis of *H. pylori* infection, using ^{13}C-urea breath test as the reference standard. Additionally, we estimated the point prevalence of *H. pylori* DNA mutations conferring resistance to clarithromycin and levofloxacin.

Research methods

We conducted a prospective two-center diagnostic test accuracy study. We enrolled consecutive people ≥ 18 years old without previous diagnosis of *H. pylori* infection, referred for dyspepsia between February and October 2017. At enrollment, all participants underwent ^{13}C-urea breath test. Participants aged over 50 years were scheduled to undergo upper endoscopy with histology. Participants collected stool samples 1-3 d after enrollment for the THD fecal test. The detection of bacterial 23S rRNA subunit gene indicated *H. pylori* infection. We also used the index diagnostic test to examine mutations conferring resistance to clarithromycin and levofloxacin. Independent investigators analyzed the index test and reference standard test results blinded to the other test findings, participants' information and histology results. We estimated diagnostic accuracy parameters, together with their 95% confidence intervals. The novelty of our research methods included an *a priori* sample size, a prospective enrollment of consecutive participants, and the blindingof outcome assessors. This approach increased the certainty of our findings.

Research results

Out of 294 participants, 95 (32.3%) had a positive ^{13}C-urea breath test. Four (1.4%) participants withdrew from the study after the enrollment visit. In the

290 participants who completed the study, the THD fecal test sensitivity was 90.2% (CI: 84.2%-96.3%), specificity 98.5% (CI: 96.8%-100%), positive predictive value 96.5% (CI: 92.6%-100%), negative predictive value 95.6% (CI: 92.8%-98.4%), accuracy 95.9% (CI: 93.6%-98.2%), positive likelihood ratio 59.5 (CI: 19.3-183.4), negative likelihood ratio 0.10 (CI: 0.05-0.18). Out of 83 *H. pylori* infected participants identified with the THD fecal test, 27 (32.5%) had bacterial strains resistant to clarithromycin, 3 (3.6%) to levofloxacin, and 4 (4.8%) to both antibiotics.

Research conclusions

Our results indicate that the THD fecal test has high diagnostic accuracy for the non-invasive diagnosis of *H. pylori* infection in patients with dyspeptic symptoms, while enabling identification of bacterium resistance to clarithromycin and levofloxacin. The certainty of our findings is based on the rigorous methodological approach used in the assessment of the THD fecal test diagnostic performance. THD fecal testing may inform clinical decision-making and guide individualized therapies to eradicate *H. pylori* infection.

Research perspectives

The spread of *H. pylori* resistance to antibiotics has prompted the investigation of the efficacy of antibiotic susceptibility-guided therapies. THD fecal testing may assist in the conduct of randomized trials to evaluate the benefits and harms of tailored eradication strategies in first-line.

ACKNOWLEDGMENTS

THD Spa, Correggio (Italy), for providing free of charge THD fecal test for all participants included in the study.

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P- Reviewer: Buzas G, Day AS, Ulaşoğlu C **S- Editor**: Wang XJ
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ISSN 1007-9327