

World Journal of *Gastroenterology*

World J Gastroenterol 2016 November 7; 22(41): 9039-9250



**EDITORIAL**

- 9039** Emerging role of obeticholic acid in the management of nonalcoholic fatty liver disease

Makri E, Cholongitas E, Tziomalos K

REVIEW

- 9044** Concise review: Interferon-free treatment of hepatitis C virus-associated cirrhosis and liver graft infection

Weiler N, Zeuzem S, Welker MW

- 9057** Mesenchymal stromal cell-based therapy: Regulatory and translational aspects in gastroenterology

Dothel G, Raschi E, Rimondini R, De Ponti F

- 9069** Genetic alterations in hepatocellular carcinoma: An update

Niu ZS, Niu XJ, Wang WH

MINIREVIEWS

- 9096** Th17 involvement in nonalcoholic fatty liver disease progression to non-alcoholic steatohepatitis

Chackelevicius CM, Gambaro SE, Tiribelli C, Rosso N

ORIGINAL ARTICLE**Basic Study**

- 9104** Dysregulation of innate immunity in ulcerative colitis patients who fail anti-tumor necrosis factor therapy

Baird AC, Mallon D, Radford-Smith G, Boyer J, Piche T, Prescott SL, Lawrance IC, Tulic MK

- 9117** Altered pattern of tumor necrosis factor-alpha production in peripheral blood monocytes from Crohn's disease

Loganes C, Pin A, Naviglio S, Girardelli M, Bianco AM, Martelossi S, Tommasini A, Piscianz E

- 9127** Esophagogastric anastomosis in rats: Improved healing by BPC 157 and L-arginine, aggravated by L-NAME

Djakovic Z, Djakovic I, Cesarec V, Madzarac G, Becejac T, Zukanovic G, Drmic D, Batelja L, Zenko Sever A, Kolenc D, Pajtak A, Knez N, Japjec M, Luetic K, Stancic-Rokotov D, Seiwert S, Sikiric P

- 9141** HER2-induced metastasis is mediated by AKT/JNK/EMT signaling pathway in gastric cancer

Choi Y, Ko YS, Park JJ, Choi Y, Kim Y, Pyo JS, Jang BG, Hwang DH, Kim WH, Lee BL

Retrospective Cohort Study

- 9154 Effect of airplane transport of donor livers on post-liver transplantation survival

Huang Y, MacQuillan G, Adams LA, Garas G, Collins M, Nwaba A, Mou L, Bulsara MK, Delriviere L, Jeffrey GP

Retrospective Study

- 9162 First-line endoscopic treatment with over-the-scope clips significantly improves the primary failure and rebleeding rates in high-risk gastrointestinal bleeding: A single-center experience with 100 cases

Richter-Schrag HJ, Glatz T, Walker C, Fischer A, Thimme R

- 9172 Presepsin teardown - pitfalls of biomarkers in the diagnosis and prognosis of bacterial infection in cirrhosis

Papp M, Tornai T, Vitalis Z, Tornai I, Tornai D, Dinya T, Sumegi A, Antal-Szalmas P

- 9186 Impact of IL28B and OAS gene family polymorphisms on interferon treatment response in Caucasian children chronically infected with hepatitis B virus

Domagalski K, Pawłowska M, Zalesna A, Pilarczyk M, Rajewski P, Halota W, Tretyn A

- 9196 Simplified criteria for diagnosing superficial esophageal squamous neoplasms using Narrow Band Imaging magnifying endoscopy

Dobashi A, Goda K, Yoshimura N, Ohya TR, Kato M, Sumiyama K, Matsushima M, Hirooka S, Ikegami M, Tajiri H

- 9205 Assessment of scoring systems for acute-on-chronic liver failure at predicting short-term mortality in patients with alcoholic hepatitis

Kim HY, Kim CW, Kim TY, Song DS, Sinn DH, Yoon EL, Jung YK, Suk KT, Lee SS, Lee CH, Kim TH, Kim JH, Yim HJ, Kim SE, Baik SK, Lee BS, Jang JY, Kim YS, Kim SG, Yang JM, Sohn JH, Lee HJ, Park SH, Choi EH, Kim DJ;
Korean Acute-on-Chronic Liver Failure Study Group

Prospective Study

- 9214 Molecular detection of *Helicobacter pylori* antibiotic resistance in stool vs biopsy samples

Brennan DE, Omorogbe J, Hussey M, Tighe D, Holleran G, O'Morain C, Smith SM, McNamara D

CASE REPORT

- 9222 Two cases of pancreatic ductal adenocarcinoma with intrapancreatic metastasis

Fujita Y, Kitago M, Masugi Y, Itano O, Shinoda M, Abe Y, Hibi T, Yagi H, Fujii-Nishimura Y, Sakamoto M, Kitagawa Y

- 9229 Collision tumor of hepatocellular carcinoma and neuroendocrine carcinoma involving the liver: Case report and review of the literature

Choi GH, Ann SY, Lee SI, Kim SB, Song IH

- 9235** Reoperation in an adult female with "right-sided" Hirschsprung's disease complicated by refractory hypertension and cough

Wei ZJ, Huang L, Xu AM

- 9242** Metabolic imaging for guidance of curative treatment of isolated pelvic implantation metastasis after resection of spontaneously ruptured hepatocellular carcinoma: A case report

Hao B, Guo W, Luo NN, Fu H, Chen HJ, Zhao L, Wu H, Sun L

Letters to The Editor

- 9247** Hepatic epithelioid hemangioendothelioma: Dilemma and challenges in the preoperative diagnosis

Hu HJ, Jin YW, Jing QY, Shrestha A, Cheng NS, Li FY

ABOUT COVER

Editorial board member of *World Journal of Gastroenterology*, Peng Hou, PhD, Professor, Department of Endocrinology, The First Affiliated Hospital of Xi'an Jiaotong University School of Medicine, Xi'an 710061, Shaanxi Province, China

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 1375 experts in gastroenterology and hepatology from 68 countries.

The primary task of *WJG* is to rapidly publish high-quality original articles, reviews, and commentaries in the fields of gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, hepatobiliary surgery, gastrointestinal oncology, gastrointestinal radiation oncology, gastrointestinal imaging, gastrointestinal interventional therapy, gastrointestinal infectious diseases, gastrointestinal pharmacology, gastrointestinal pathophysiology, gastrointestinal pathology, evidence-based medicine in gastroenterology, pancreatology, gastrointestinal laboratory medicine, gastrointestinal molecular biology, gastrointestinal immunology, gastrointestinal microbiology, gastrointestinal genetics, gastrointestinal translational medicine, gastrointestinal diagnostics, and gastrointestinal therapeutics. *WJG* is dedicated to become an influential and prestigious journal in gastroenterology and hepatology, to promote the development of above disciplines, and to improve the diagnostic and therapeutic skill and expertise of clinicians.

INDEXING/ABSTRACTING

World Journal of Gastroenterology (*WJG*) is now indexed in Current Contents[®]/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch[®]), Journal Citation Reports[®], Index Medicus, MEDLINE, PubMed, PubMed Central, Digital Object Identifier, and Directory of Open Access Journals. The 2015 edition of Journal Citation Reports[®] released by Thomson Reuters (ISI) cites the 2015 impact factor for *WJG* as 2.787 (5-year impact factor: 2.848), ranking *WJG* as 38 among 78 journals in gastroenterology and hepatology (quartile in category Q2).

FLYLEAF

I-IX Editorial Board

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*
Responsible Electronic Editor: *Cai-Hong Wang*
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Jing Yu*
Proofing Editorial Office Director: *Jin-Lei Wang*

NAME OF JOURNAL
World Journal of Gastroenterology

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

LAUNCH DATE
October 1, 1995

FREQUENCY
Weekly

EDITORS-IN-CHIEF
Damian Garcia-Olmo, MD, PhD, Doctor, Professor, Surgeon, Department of Surgery, Universidad Autonoma de Madrid; Department of General Surgery, Fundacion Jimenez Diaz University Hospital, Madrid 28040, Spain

Stephen C Strom, PhD, Professor, Department of Laboratory Medicine, Division of Pathology, Karolinska Institutet, Stockholm 141-86, Sweden

Andrzej S Tarnawski, MD, PhD, DSc (Med), Professor of Medicine, Chief Gastroenterology, VA Long Beach Health Care System, University of California, Irvine, Irvine, CA, 5901 E. Seventh Str., Long Beach, CA 90822, United States

fornia, Irvine, CA, 5901 E. Seventh Str., Long Beach, CA 90822, United States

EDITORIAL BOARD MEMBERS
All editorial board members resources online at <http://www.wjgnet.com/1007-9327/editorialboard.htm>

EDITORIAL OFFICE
Jin-Lei Wang, Director
Yuan Qi, Vice Director
World Journal of Gastroenterology
Baishideng Publishing Group Inc
8226 Regency Drive,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>
<http://www.wjgnet.com>

PUBLISHER
Baishideng Publishing Group Inc
8226 Regency Drive,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>

PUBLICATION DATE
November 7, 2016

COPYRIGHT
© 2016 Baishideng Publishing Group Inc. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

INSTRUCTIONS TO AUTHORS
Full instructions are available online at <http://www.wjgnet.com/bpg/gerinfo/204>

ONLINE SUBMISSION
<http://www.wjgnet.com/esps/>

Prospective Study

Molecular detection of *Helicobacter pylori* antibiotic resistance in stool vs biopsy samples

Denise E Brennan, Joseph Omorogbe, Mary Hussey, Donal Tighe, Grainne Holleran, Colm O'Morain, Sinéad M Smith, Deirdre McNamara

Denise E Brennan, Joseph Omorogbe, Mary Hussey, Donal Tighe, Grainne Holleran, Colm O'Morain, Sinéad M Smith, Deirdre McNamara, Department of Clinical Medicine, School of Medicine, Trinity College Dublin, Dublin 2, Ireland

Sinéad M Smith, School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Dublin 2, Ireland

Author contributions: Smith SM and McNamara D contributed equally this work; Brennan DE, Omorogbe J, Hussey M, Tighe D, Holleran G, O'Morain C and McNamara D recruited patients and collected samples for the study; Brennan DE and Smith SM conducted experiments, analysed data and wrote the manuscript; all authors critically reviewed and approved the manuscript prior to publication.

Institutional review board statement: The study was reviewed and approved by the Adelaide and Meath Hospital Research Ethics Committee.

Conflict-of-interest statement: No potential conflicts of interest relevant to this article were reported.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Correspondence to: Dr. Sinéad M Smith, PhD, Assistant Professor, Department of Clinical Medicine, School of Medicine, Trinity College Dublin, Tallaght, Dublin 2, Ireland. smithsi@tcd.ie
Telephone: +35-318-961385
Fax: +35-318-962988

Received: August 9, 2016

Peer-review started: August 11, 2016

First decision: September 12, 2016

Revised: September 27, 2016

Accepted: October 19, 2016

Article in press: October 19, 2016

Published online: November 7, 2016

Abstract

AIM

To compare (1) demographics in urea breath test (UBT) vs endoscopy patients; and (2) the molecular detection of antibiotic resistance in stool vs biopsy samples.

METHODS

Six hundred and sixteen adult patients undergoing endoscopy or a UBT were prospectively recruited to the study. The GenoType HelicoDR assay was used to detect *Helicobacter pylori* (*H. pylori*) and antibiotic resistance using biopsy and/or stool samples from CLO-positive endoscopy patients and stool samples from UBT-positive patients.

RESULTS

Infection rates were significantly higher in patients referred for a UBT than endoscopy (overall rates: 33% vs 19%; treatment-naïve patients: 33% vs 14.7%, respectively). *H. pylori*-infected UBT patients were younger than *H. pylori*-infected endoscopy patients (41.4 vs 48.4 years, respectively, $P < 0.005$), with a higher percentage of *H. pylori*-infected males in the endoscopy-compared to the UBT-cohort (52.6% vs 33.3%, $P = 0.03$). The GenoType HelicoDR assay was more accurate at detecting *H. pylori* infection using biopsy samples than stool samples [98.2% ($n = 54/55$) vs 80.3% ($n = 53/66$), $P < 0.005$]. Subset analysis using stool and biopsy samples from CLO-positive endoscopy patients revealed a higher detection rate of

resistance-associated mutations using stool samples compared to biopsies. The concordance rates between stool and biopsy samples for the detection of *H. pylori* DNA, clarithromycin and fluoroquinolone resistance were just 85%, 53% and 35%, respectively.

CONCLUSION

Differences between endoscopy and UBT patients provide a rationale for non-invasive detection of *H. pylori* antibiotic resistance. However, the GenoType HelicoDR assay is an unsuitable approach.

Key words: *Helicobacter pylori*; Antibiotic resistance; Clarithromycin; Fluoroquinolone; Molecular methods

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: The successful detection of clarithromycin and/or fluoroquinolone resistant *Helicobacter pylori* (*H. pylori*) infections by non-invasive methods would enable a widespread assessment of resistance rates. Here we evaluate the GenoType HelicoDR assay for the detection of clarithromycin and fluoroquinolone resistance using DNA isolated from stool samples compared to biopsy samples. Although results using this assay on biopsy tissue have previously been shown to correspond well with culture and antimicrobial susceptibility testing, there was weak correlation between results obtained using biopsy vs stool samples in our study. Further studies are required to optimise the non-invasive detection of clarithromycin and fluoroquinolone resistant *H. pylori* infection.

Brennan DE, Omorogbe J, Hussey M, Tighe D, Holleran G, O'Morain C, Smith SM, McNamara D. Molecular detection of *Helicobacter pylori* antibiotic resistance in stool vs biopsy samples. *World J Gastroenterol* 2016; 22(41): 9214-9221 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v22/i41/9214.htm> DOI: <http://dx.doi.org/10.3748/wjg.v22.i41.9214>

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a gram-negative bacterium that specifically colonizes the epithelium of the human stomach, in particular the gastric antrum. It infects approximately 50% of the world's population. The prevalence of *H. pylori* varies globally, increasing with older age and lower socio-economic status. Most infected individuals will not develop any clinically significant complications; however the most common symptoms of infection are gastritis and gastric or duodenal ulcers. The diagnosis and treatment of *H. pylori* infection are critical factors in the prevention and management of these conditions^[1-3]. *H. pylori* infection can be detected by invasive and non-invasive means,

using a variety of diagnostic tests. The Maastricht IV/Florence Consensus Report recommends the "Test and Treat" strategy for patients presenting with uncomplicated dyspepsia with no alarm symptoms associated with an increased risk of gastric cancer^[2]. In the Irish healthcare setting, the urea breath test (UBT) is the current gold standard non-invasive test for *H. pylori* infection in patients managed by the "Test and Treat" strategy. The UBT is highly accurate with a sensitivity of 88%-95% and specificity of 95%-100%^[4]. For patients presenting with new onset dyspepsia (above 45 years; European guidelines) or dyspepsia along with accompanying alarm symptoms such as weight loss, gastrointestinal bleeding, abdominal mass or iron deficient anaemia, endoscopy is recommended^[2]. When an endoscopy is performed, *H. pylori* infection can be diagnosed using gastric biopsy specimens. The most common test employed is the rapid urease-test for *Campylobacter*-like organisms (CLO), which has a sensitivity of > 90% and specificity of > 95%^[5].

Treatment for *H. pylori* infection is recommended in all symptomatic individuals. However, eradication rates have fallen in many countries in recent years^[6-8] mainly due to poor patient compliance and the emergence of antibiotic resistant strains of *H. pylori*, particularly to clarithromycin and levofloxacin^[9-11]. The European *Helicobacter* and Microbiota Study Group (EHMSG) and the most recent Maastricht IV/Florence Consensus recommend local surveillance of existing and emerging antibiotic resistance and that the combination of antibiotics for *H. pylori* eradication should be chosen according to the local resistance patterns^[2,10]. Clarithromycin-based first-line triple therapy is no longer recommended in regions where antibiotic resistance surveillance indicates that clarithromycin resistance is above 15%-20%^[2]. Since *H. pylori* is a fastidious bacterium, culture and antimicrobial sensitivity testing is time-consuming. The sensitivity of culture of *H. pylori* from gastric biopsy samples has been reported to be as low as 55%^[11]. Molecular testing represents an attractive alternative to culture-based methods and has been recommended by the Maastricht Consensus guidelines to detect *H. pylori* and both clarithromycin and fluoroquinolone resistance when standard culture and sensitivity testing are unavailable^[2]. Single point mutations (most commonly A2146C, A2146G and A2147G) within the *H. pylori* *rrl* gene that encodes the 23S ribosomal subunit confer clarithromycin resistance^[11,12]. The most significant mutations conferring fluoroquinolone resistance are located at positions 87 (N87K) and 91 (D91N, D91G, D91Y) of the *H. pylori* *gyrA* gene, which encodes the A subunit of the DNA gyrase enzyme^[11,12]. The GenoType HelicoDR assay allows for the molecular genetic identification of *H. pylori* and its resistance to clarithromycin and fluoroquinolones, such as levofloxacin. The assay has been reported to be efficient at detecting mutations predictive of antibiotic resistance when applied to *H. pylori* cultures or gastric biopsy

specimens^[13-16], with a sensitivity and specificity of 94%-100% and 86%-99% for detecting clarithromycin resistance and 83%-87% and 95%-98.5% for detecting fluoroquinolone resistance, respectively^[16,17].

Currently, *H. pylori* antibiotic resistance surveillance is based primarily on patients undergoing invasive testing by means of endoscopy. However, most patients are diagnosed by non-invasive methods such as the UBT. As such, antibiotic resistance data obtained solely from endoscopy patients may not reflect the true prevalence of *H. pylori* infection and the rates of antibiotic resistance in symptomatic patients. The aims of this study were to (1) compare demographics and prevalence of *H. pylori* infection in patients referred for endoscopy with those of patients referred for a UBT; and (2) evaluate the potential use of the GenoType HelicoDR assay for the non-invasive detection of *H. pylori* and antibiotic resistant infection using stool samples.

MATERIALS AND METHODS

Study design and ethics

A prospective study was carried out in a tertiary referral teaching hospital (Adelaide and Meath Hospital, Dublin, Ireland) affiliated with Trinity College Dublin. Patients who had been referred to the endoscopy clinic were included from August 2014 until March 2016. The study received ethical approval from the Adelaide and Meath Hospital Research Ethics Committee. Informed consent was obtained from all patients before enrolment.

Study population

Inclusion criteria were (1) ability and willingness to participate in the study and to provide informed consent; and (2) confirmed *H. pylori* infection by UBT or a positive rapid urease test (TRI-MED Distributors, PTY LTD, Washington, United States) at 60 min performed and/or histology.

Exclusion criteria were (1) age less than 18 years; (2) pregnancy or lactation; (3) severe inter-current illness; (4) current PPI use or recent antibiotic use (within 4 wk); and (5) bleeding problems or use of blood thinning drugs (for endoscopy patients).

Sample collection and antimicrobial susceptibility genotyping

A single corpus and antrum biopsy from each patient was placed into DENT transport medium (brain heart infusion broth containing 2.5% (w/v) yeast extract, 5% sterile horse serum and Dent *Helicobacter* Selective Supplement; Oxoid, Basingstoke, United Kingdom) for transport to the research laboratory. Biopsies were placed into fresh collection tubes and stored at -20 °C until processed for genomic DNA isolation using the QIAamp DNA Mini Kit (Qiagen GmbH, Hilden, Germany) according to manufacturer's instructions. Patients attending for endoscopy or the UBT were invited to provide a stool sample collected within 24 h of their appointment. Stool samples were stored at 4 °C until processed

for genomic DNA isolation using the PSP Spin Stool DNA Plus Kit (STRATEC Molecular GmbH, Berlin, Germany) according to the manufacturers' instructions. All isolated DNA was stored -20 °C until genotyping for clarithromycin and fluoroquinolone-mediating mutations was performed using the GenoType HelicoDR assay (Hain Lifescience GmbH, Nehren, Germany). Multiplex amplification of DNA regions of interest was performed using the biotinylated primers supplied in the GenoType HelicoDR kit and the Hotstart Taq DNA polymerase kit (Qiagen). PCR products were reverse hybridised to DNA strips containing probes for gene regions of interest, developed and interpreted according to the manufacturers' instructions. Briefly, all strips were analysed for the presence of a conjugate control band (to indicate successful conjugate binding and substrate reaction), an amplification control band (to indicate a successful amplification reaction), a *H. pylori* control band (to document the presence of a *H. pylori* strain) and gene locus control bands for *gyrA* and 23S (to indicate successful detection of the gene regions of interest). In addition, the strips were analysed for the presence of wild type and/or mutation bands. An infection was considered clarithromycin sensitive when the 23S wild-type probe stained positive and clarithromycin resistant if one of the 23S mutation probes stained positive. As per manufacturers' instructions, results of both positions of the *gyrA* gene were combined to draw conclusions about fluoroquinolone resistance. Thus, an infection was only considered fluoroquinolone sensitive when one of the wild-type probes for codon 87 of the *gyrA* gene stained positive together with a positive wild-type probe for codon 91. Fluoroquinolone resistance was indicated if either the wild-type probes for codon 87 or the wild-type probe for codon 91 stained negative, or if one of the mutant codon 87 or 91 probes stained positive. For all mutations probes, only bands whose intensities were equal to or stronger than the amplification control were considered positive.

Statistical analysis

Statistical analysis was carried out using GraphPad Prism (GraphPad Software Inc., CA, United States). Continuous variables are presented as arithmetic mean and SD. *P* values for continuous variables were calculated and compared using the two-tailed independent *t*-test. Categorical variables are presented as percentages and 95% confidence intervals (95%CI). *P* values for categorical variables were calculated using the Fisher's exact test/Pearson χ^2 test. In all cases, a *P* value less than 0.05 was considered significant.

RESULTS

Prevalence of *H. pylori* infection and demographics of endoscopy and UBT patients

A schematic of patient inclusion and analysis is presented in Figure 1. In all, 616 patients were

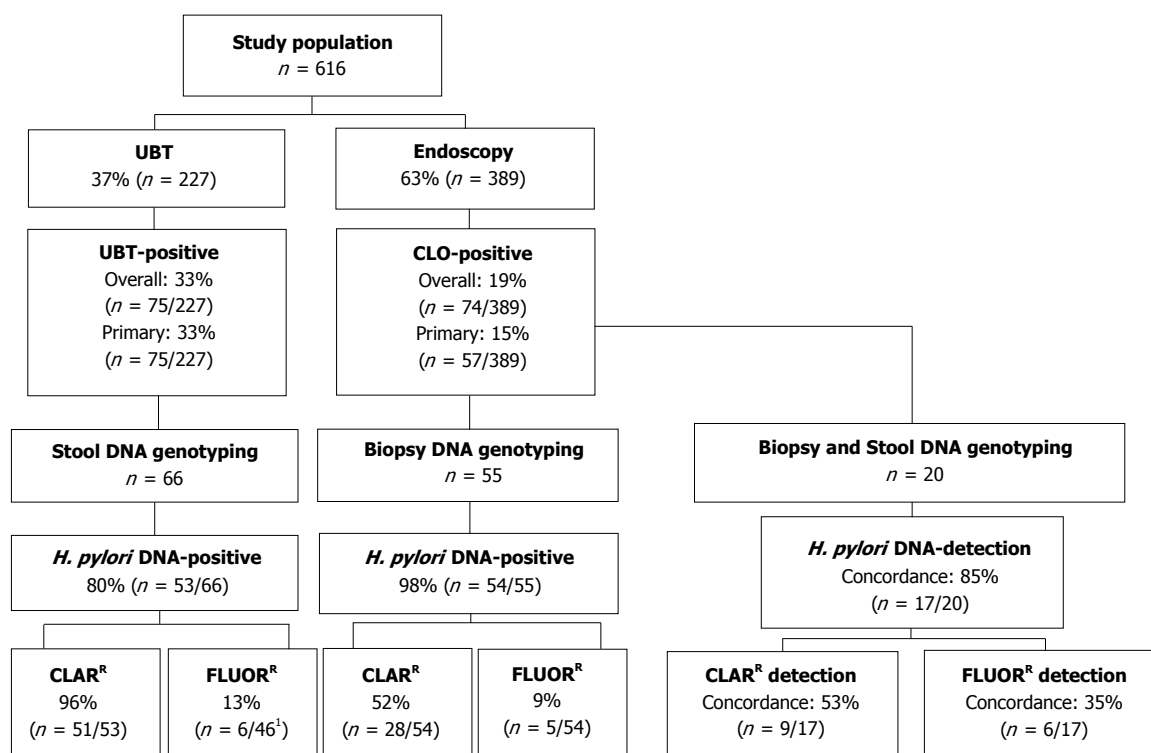


Figure 1 Flow chart of patient inclusion and analysis. ¹Only samples that were positive for the control gene locus for the fluoroquinolone resistance gene were included. CLAR^R: Clarithromycin resistant; FLUOR^R: Fluoroquinolone resistant.

Table 1 Demographics of endoscopy and urea breath test patients

	Endoscopy	UBT	P value	95%CI
Total	n = 389	n = 227		
Mean age (SD)	52.3 (16.4)	39.6 (12.6)	< 0.001	10.22-15.18
Male	42.2% (n = 164)	30.4% (n = 69)	< 0.005	3.68-19.60
Primary <i>H. pylori</i> infection	14.7% (n = 57)	33.0% (n = 75)	< 0.001	11.07-25.65
Mean age (SD)	48.4 (14.9)	41.4 (13.0)	< 0.005	2.19-11.81
Male	52.6% (n = 30)	33.3% (n = 25)	0.03	1.23-36.29

included in the study between August 2014 and March 2016; 389 patients (mean age 52.3 years, 42.2% male) underwent endoscopy and 227 patients (mean age 39.6 years, 30.4% male) a UBT (Table 1). The overall prevalence of *H. pylori* infection was significantly higher in the UBT cohort than the endoscopy cohort [33.0% (n = 75) vs 19% (n = 74), $P < 0.001$; 95%CI: 6.58-21.54] (Figure 1). Of the *H. pylori*-positive endoscopy patients (CLO-positive), 17 had been previously treated for *H. pylori* infection, therefore the prevalence of primary *H. pylori* infection was 14.7% (n = 57). All of the *H. pylori*-positive UBT patients were treatment naïve, thus the prevalence of primary *H. pylori* infection was also significantly higher in patients referred for UBT than for endoscopy (33.0% vs 14.7%, $P < 0.001$, 95%CI: 11.07-25.65; Figure 1 and Table 1). In keeping with the guidelines recommending endoscopy for symptomatic patients over 45 years, *H. pylori*-positive patients in the endoscopy cohort were significantly older than those in the UBT cohort (48.4 years vs 41.4 years; $P < 0.005$,

95%CI: 2.19-11.81). There were a greater number of *H. pylori*-positive men in the endoscopy cohort than the UBT cohort (52.6% vs 33.3%, $P = 0.03$, 95%CI: 1.23-36.29; Table 1). Taken together, these findings indicate significant differences in demographics and the prevalence of both overall and primary *H. pylori* infection rates in patients referred for endoscopy and those referred for the UBT.

Comparison of *H. pylori* detection and the prevalence of antibiotic resistance-mediating mutations using the GenoType HelicoDR assay in endoscopy vs UBT patients using biopsies and stool samples, respectively

The GenoType HelicoDR assay is based on DNA strip technology that enables the molecular genetic identification of *H. pylori* and resistance to fluoroquinolones and/or clarithromycin by detecting the most frequent mutations in the *gyrA* gene (codons 87 and 91) and the 23S gene (positions 2146 and 2147), respectively. Previous studies have demonstrated strong correlations between results obtained using the GenoType

Table 2 Molecular detection of *Helicobacter pylori* infection and resistance-mediating mutations using biopsies from CLO-positive endoscopy patients and stool samples of Urea Breath Test-positive patients

	Biopsy Specimens	Stool specimens	P value	95%CI
Total analysed	<i>n</i> = 55	<i>n</i> = 66		
<i>H. pylori</i> DNA positive	98.2% (<i>n</i> = 54/55)	80.3 % (<i>n</i> = 53/66)	< 0.005	6.10-29.66
Clarithromycin resistant	51.9% (<i>n</i> = 28/54)	96.2 % (<i>n</i> = 51/53)	< 0.001	27.70-58.71
Fluoroquinolone resistant	9.3 % (<i>n</i> = 5/54) ¹	13 % (<i>n</i> = 6/46) ¹	0.55	-9.99-18.28

¹Fluoroquinolone and clarithromycin mutations present. *H. pylori*: *Helicobacter pylori*.

Table 3 Concordance in the detection of *Helicobacter pylori* and antibiotic resistance-mediating mutations between results obtained using biopsies vs stool samples from individual *Campylobacter*-like organisms-positive endoscopy patients

	Biopsy Specimens	Stool specimens	Concordance
Total analysed	<i>n</i> = 20	<i>n</i> = 20	
<i>H. pylori</i> DNA positive	95% (<i>n</i> = 19/20)	90% (<i>n</i> = 18/20)	85% (<i>n</i> = 17/20)
Clarithromycin resistant ¹	52.9% (<i>n</i> = 9/17)	100.0% (<i>n</i> = 17/17)	52.9% (<i>n</i> = 9/17)
Fluoroquinolone resistant ¹	23.5% (<i>n</i> = 4/17)	52.9% (<i>n</i> = 9/17)	35.3% (<i>n</i> = 6/17)

¹Only samples where *H. pylori* DNA was detected were analysed. *H. pylori*: *Helicobacter pylori*.

HelicoDR assay on biopsy specimens compared to culture and antimicrobial testing^[14,16,17]. In order to evaluate the GenoType HelicoDR assay for the non-invasive detection of *H. pylori* using stool samples, we first set out to compare the detection rate of *H. pylori* infection using stool samples from *H. pylori*-positive UBT patients with that obtained using biopsy samples from CLO-positive endoscopy patients. Initial control experiments showed that the assay did not detect *H. pylori* DNA in stool samples from 2 uninfected UBT-negative patients (not shown). In *H. pylori*-infected patients, the GenoType HelicoDR assay was significantly more accurate at detecting *H. pylori* infection using biopsy samples than stool samples [98.2% (*n* = 54/55) vs 80.3% (*n* = 53/66), *P* < 0.005, 95%CI: 6.10-29.66] (Figure 1 and Table 2).

Next, the prevalence of antibiotic resistance-mediating mutations was compared using stool samples from *H. pylori*-positive UBT patients and biopsy samples from CLO-positive endoscopy patients. Using the GenoType HelicoDR assay, the 23S gene locus control was positive in all of *H. pylori*-positive DNA samples isolated from either biopsy tissue (100%, *n* = 54/54) or stool specimens (100%, *n* = 53/53). A significantly higher level of clarithromycin resistance-mediating mutations was detected using stool samples from UBT-positive patients than biopsy samples from CLO-positive patients [96.2% (*n* = 51/53) vs 51.9% (*n* = 28/54), *P* < 0.001, 95%CI: 27.70-58.71] (Figure 1 and Table 2).

In terms of *gyrA* genotyping, the *gyrA* locus control probe was positive in all DNA samples isolated from biopsy tissue (100%, *n* = 54/54), but only 86.8% (*n* = 46/53) of *H. pylori*-positive DNA samples isolated from stool. Fluoroquinolone resistance-mediating mutations were detected in 9.3% (*n* = 5/54) of biopsy samples from CLO-positive patients compared to 13% (*n* =

6/46) of stool samples from UBT-positive patients (*P* = 0.56, 95%CI: -9.99-18.28; Figure 1 and Table 2). For both endoscopy and UBT patients, all samples that were positive for fluoroquinolone resistance mutations were positive for clarithromycin resistance mutations (Table 2). Taken together, these findings indicate that the GenoType HelicoDR assay is more accurate at detecting *H. pylori* DNA using biopsies from CLO-positive endoscopy patients than stool DNA isolated from UBT-positive patients. In addition, the assay detected a significantly higher rate of clarithromycin resistance using stool samples from patients diagnosed by the UBT than that obtained when biopsy samples from CLO-positive endoscopy patients were analysed.

Evaluation of the GenoType HelicoDR assay for the detection of resistance-mediating mutations by comparing stool and biopsy analyses from individual patients

Given the high rate of clarithromycin resistance detected using stool specimens from UBT-positive patients (96.2%; Table 2) and the lack of published data on the use of the GenoType HelicoDR assay for stool sample analysis, we next set out to directly compare a stool DNA sample with that of a biopsy DNA sample isolated from a subset of the CLO-positive endoscopy patients. In all, stool and biopsy samples from 20 CLO-positive patients were analysed (mean age 46.8 ± 15.8 years, 50% male). *H. pylori* DNA was detected in 95% (*n* = 19/20) of biopsy samples and 90% (*n* = 18/20) of stool samples from the CLO-positive patients. Concordance between results from biopsy and stool samples of individual patients for the detection of *H. pylori* DNA was 85% (*n* = 17/20; Figure 1 and Table 3). In terms of antibiotic resistance, results were compared in the 17 patients with concordant results for the presence of *H. pylori* DNA in

both their stool and biopsy samples. Concordance for the analysis of stool and biopsy samples of individual patients was just 52.9% ($n = 9/17$) for clarithromycin resistance and 35.3% ($n = 6/17$) for fluoroquinolone resistance (Figure 1, Table 3). Higher rates of both clarithromycin and fluoroquinolone resistance were detected in stool samples compared to biopsy samples obtained from the same patient (Table 3), suggesting a lack of specificity of the assay for the detection of antibiotic resistance-mediating mutations using DNA isolated from stool samples.

DISCUSSION

As the recommended first-line therapy for *H. pylori* infection should be guided by the local prevalence of primary clarithromycin resistance and third-line and subsequent treatment regimens should be guided by antimicrobial susceptibility testing^[2], methods for detecting antibiotic resistance are of great interest. Antimicrobial susceptibility testing for *H. pylori* is mainly performed using biopsy specimens obtained by invasive means at endoscopy. As a result, findings on the prevalence of *H. pylori* infection and antibiotic resistance based solely on this patient cohort may not represent the true rates of resistance in a given population. In order to determine whether *H. pylori*-infected endoscopy patients are representative of the wider *H. pylori*-infected population, we compared the prevalence of infection and patient demographics between endoscopy patients with those referred for non-invasive *H. pylori* diagnoses by the UBT. Indeed we found significant differences between the two patient cohorts. Both the overall infection rate and the prevalence of primary infection in *H. pylori* treatment-naïve patients were significantly higher in patients referred for a UBT than endoscopy (overall infection rates of 33% vs 19% respectively, and primary infection rates of 33% vs 14.7%, respectively). *H. pylori*-infected UBT patients were also significantly younger than *H. pylori*-infected endoscopy patients (41.4 vs 48.4 years, respectively), with a higher percentage of *H. pylori* infected males in the endoscopy compared to UBT cohort (52.6% vs 33.3%). Both age and sex have been reported as risk factors for *H. pylori* antibiotic resistance, for example age > 50 years has been reported as a risk factor for levofloxacin resistance and being female has been associated with metronidazole resistance in the most recent pan-European study on antimicrobial resistance^[10]. Thus the statistically significant differences in age and sex between endoscopy and UBT patients in our study suggests that *H. pylori*-infected endoscopy patients are likely not representative of the wider *H. pylori*-positive cohort, providing a strong rationale for performing more widespread antimicrobial susceptibility testing.

Successfully extending molecular-based methods to diagnose *H. pylori* non-invasively would greatly enhance our ability to more accurately assess the

prevalence of resistance to a range of antibiotics, and enable clinicians to offer personalised antimicrobial susceptibility-based therapy to a wider number of patients. *H. pylori* DNA has been detected in a number of clinical specimens including blood, stool samples and oral cavity specimens^[18-22]. Analysis of stool samples has shown the most promise for the molecular detection of clarithromycin resistance-mediating mutations to date^[18,23-28]. Studies have demonstrated sensitivity and specificity values of 83%-98% and 98%-100%, respectively, for the detection clarithromycin resistance using the *H. pylori* ClariRes Assay (Ingenetix) to analyse stool samples^[23-26]. However, data on the detection of *H. pylori* fluoroquinolone resistance using stool samples is lacking. Although the GenoType HelicoDR assay has proven useful for detecting clarithromycin and fluoroquinolone resistance in biopsy or culture specimens^[13-17], evaluation of the assay for the analysis of stool specimens presented herein proved suboptimal. Firstly, the assay detected *H. pylori* infection in a significantly lower percentage of *H. pylori*-infected patients when stool rather than biopsy specimens were analysed (80%-90% vs 95%-98.2%; respectively Tables 2 and 3). As *H. pylori* specifically colonizes the stomach and is not an intestinal bacterium, it is present only in low numbers in the stool, a factor which may have impacted the sensitivity of *H. pylori* detection using stool samples in our study. Additionally, *H. pylori* DNA may be exposed to enzymatic or mechanical degradation during transit from the stomach through the intestines^[22]. When results using biopsy samples from individual *H. pylori*-infected patients were directly compared with those obtained from their stool samples, concordance scores for clarithromycin and fluoroquinolone resistance were just 52.9% and 35.6%, respectively. In addition, a higher rate of clarithromycin and fluoroquinolone resistance was detected in DNA isolated from the stool samples compared to DNA isolated from biopsy samples from the same patient (Table 3). Given that previous studies have demonstrated strong correlations between results obtained using the GenoType HelicoDR assay on biopsy specimens compared to culture and antimicrobial testing^[14,16,17], this would suggest that stool sample analysis using the GenoType HelicoDR assay is less sensitive than biopsy sample analysis, providing an explanation for the high rates of antibiotic resistance obtained using stool samples from the UBT patients in Table 2. The presence of large amounts of diverse commensal bacteria in the stool may hamper the specificity of the Genotype HelicoDR assay in detection of *H. pylori* antibiotic resistance-mediating mutations. Our findings suggest it is currently unsuitable for the accurate detection of clarithromycin and fluoroquinolone resistance-mediating mutations in stool specimens. Further studies are required to extend approaches for the non-invasive detection of *H. pylori* resistance to include multiple antibiotics. Recent advances in next generation DNA sequencing technologies may provide

more robust opportunities for the accurate analysis of specific resistance-associated DNA regions. The successful optimisation of molecular-based antimicrobial susceptibility testing methods will enable resistance data obtained from patients managed by the "Test and Treat" strategy to be utilised in choosing effective antibiotics for the treatment of *H. pylori*. In this way, eradication rates for *H. pylori* may be improved.

COMMENTS

Background

Currently antimicrobial susceptibility testing for *Helicobacter pylori* (*H. pylori*) is mainly performed using cultures isolated from tissue biopsy samples obtained at endoscopy by invasive means. However, many patients are diagnosed with *H. pylori* infection by non-invasive means, such as the urea breath test. As such, antibiotic resistance data based solely on endoscopy patients may not truly reflect the prevalence of antibiotic resistance in the wider *H. pylori* infected population.

Research frontiers

Molecular methods for the detection of *H. pylori* antibiotic resistance-mediating mutations offer a more rapid alternative to standard culture-based methods. Studies have shown that data generated using molecular methods on tissue biopsy samples correlates well with culture and antimicrobial susceptibility testing. Data on the use of molecular methods, in particular the GenoType HelicoDR assay, for the analysis of stool samples is limited.

Innovations and breakthroughs

The present findings suggest that the GenoType HelicoDR assay is not suitable for the accurate detection of antibiotic resistance-mediating mutations using stool samples from *H. pylori* infected patients. Alternative PCR or DNA sequencing-based methods may show more potential.

Applications

While the GenoType HelicoDR assay has been shown to be accurate for the analysis of clarithromycin- and fluoroquinolone-mediating mutations using biopsy tissue samples, the present findings indicate that this assay is not suitable for the analysis of stool samples.

Peer-review

The authors described an examination of antibiotic resistance in both gastric biopsy and stool samples obtained from patients who underwent testing for a urea breath test or had a gastroscopy performed. The main conclusion is that the GenoType HelicoDR assay is not appropriate for use on stool samples. This seriously limits its use and thus the paper is of importance and deserves to be published. It would have been useful to include formal sensitivity testing to the bacteria isolated on gastric biopsy.

REFERENCES

- 1 **McColl KE.** Clinical practice. *Helicobacter pylori* infection. *N Engl J Med* 2010; **362**: 1597-1604 [PMID: 20427808 DOI: 10.1056/NEJMcP1001110]
- 2 **Malfertheiner P, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, Gensini GF, Gisbert JP, Graham DY, Rokkas T, El-Omar EM, Kuipers EJ.** Management of *Helicobacter pylori* infection--the Maastricht IV/ Florence Consensus Report. *Gut* 2012; **61**: 646-664 [PMID: 22491499 DOI: 10.1136/gutjnl-2012-302084]
- 3 **Smith SM, Haider RB, O'Connor H, McNamara D, O'Morain C.** Practical treatment of *Helicobacter pylori*: a balanced view in changing times. *Eur J Gastroenterol Hepatol* 2014; **26**: 819-825 [PMID: 24892516 DOI: 10.1097/MEG.000000000000130]
- 4 **Howden CW, Hunt RH.** Guidelines for the management of *Helicobacter pylori* infection. Ad Hoc Committee on Practice Parameters of the American College of Gastroenterology. *Am J Gastroenterol* 1998; **93**: 2330-2338 [PMID: 9860388 DOI: 10.1111/j.1572-0241.1998.00684.x]
- 5 **Gisbert JP, Pajares JM.** Review article: 13C-urea breath test in the diagnosis of *Helicobacter pylori* infection -- a critical review. *Aliment Pharmacol Ther* 2004; **20**: 1001-1017 [PMID: 15569102 DOI: 10.1111/j.1365-2036.2004.02203.x]
- 6 **Malfertheiner P, Bazzoli F, Delchier JC, Celiński K, Giguère M, Rivière M, Mégraud F.** *Helicobacter pylori* eradication with a capsule containing bismuth subcitrate potassium, metronidazole, and tetracycline given with omeprazole versus clarithromycin-based triple therapy: a randomised, open-label, non-inferiority, phase 3 trial. *Lancet* 2011; **377**: 905-913 [PMID: 21345487 DOI: 10.1016/S0140-6736(11)60020-2]
- 7 **Haider RB, Brennan DE, Omorogbe J, Holleran G, Hall B, O'Morain C, Breslin N, O'Connor HJ, Smith SM, McNamara D.** A randomized-controlled study to compare the efficacy of sequential therapy with standard triple therapy for *Helicobacter pylori* eradication in an Irish population. *Eur J Gastroenterol Hepatol* 2015; **27**: 1265-1269 [PMID: 26287955 DOI: 10.1097/MEG.0000000000000457]
- 8 **Smith SM.** An update on the treatment of *Helicobacter pylori* infection. *EMJ Gastroenterology* 2015; **4**: 101-107
- 9 **Graham DY, Fischbach L.** *Helicobacter pylori* treatment in the era of increasing antibiotic resistance. *Gut* 2010; **59**: 1143-1153 [PMID: 20525969 DOI: 10.1136/gut.2009.192757]
- 10 **Megraud F, Coenen S, Versporten A, Kist M, Lopez-Brea M, Hirschl AM, Andersen LP, Goossens H, Glupczynski Y.** *Helicobacter pylori* resistance to antibiotics in Europe and its relationship to antibiotic consumption. *Gut* 2013; **62**: 34-42 [PMID: 22580412 DOI: 10.1136/gutjnl-2012-302254]
- 11 **Smith SM, O'Morain C, McNamara D.** Antimicrobial susceptibility testing for *Helicobacter pylori* in times of increasing antibiotic resistance. *World J Gastroenterol* 2014; **20**: 9912-9921 [PMID: 25110421 DOI: 10.3748/wjg.v20.i29.9912]
- 12 **Mégraud F, Bénéjat L, Ontsira Ngoyi EN, Lehours P.** Molecular Approaches to Identify *Helicobacter pylori* Antimicrobial Resistance. *Gastroenterol Clin North Am* 2015; **44**: 577-596 [PMID: 26314669 DOI: 10.1016/j.gtc.2015.05.002]
- 13 **Vannarath S, Vilaichone RK, Rasachak B, Mairiang P, Yamaoka Y, Mahachai V.** Antibiotic Resistant Pattern of *Helicobacter Pylori* Infection Based on Molecular Tests in Laos. *Asian Pac J Cancer Prev* 2016; **17**: 285-287 [PMID: 26838225]
- 14 **Lee JW, Kim N, Nam RH, Park JH, Choi YJ, Kim JM, Kim JS, Jung HC.** GenoType HelicoDR test in the determination of antimicrobial resistance of *Helicobacter pylori* in Korea. *Scand J Gastroenterol* 2014; **49**: 1058-1067 [PMID: 24957849 DOI: 10.3109/00365521.2014.894117]
- 15 **Tanhi NF, Ndip RN.** Molecular Detection of Antibiotic Resistance in South African Isolates of *Helicobacter pylori*. *Gastroenterol Res Pract* 2013; **2013**: 259457 [PMID: 23710166 DOI: 10.1155/2013/259457]
- 16 **Miendje Deyi VY, Burette A, Bentatou Z, Maaroufi Y, Bontems P, Lepage P, Reynders M.** Practical use of GenoType® HelicoDR, a molecular test for *Helicobacter pylori* detection and susceptibility testing. *Diagn Microbiol Infect Dis* 2011; **70**: 557-560 [PMID: 21696906 DOI: 10.1016/j.diagmicrobio.2011.05.002]
- 17 **Cambau E, Allerheiligen V, Coulon C, Corbel C, Lascols C, Deforges L, Soussy CJ, Delchier JC, Megraud F.** Evaluation of a new test, genotype HelicoDR, for molecular detection of antibiotic resistance in *Helicobacter pylori*. *J Clin Microbiol* 2009; **47**: 3600-3607 [PMID: 19759218 DOI: 10.1128/JCM.00744-09]
- 18 **Rimbara E, Sasatsu M, Graham DY.** PCR detection of *Helicobacter pylori* in clinical samples. *Methods Mol Biol* 2013; **943**: 279-287 [PMID: 23104297 DOI: 10.1007/978-1-60327-353-4_19]
- 19 **Mentis A, Lehours P, Mégraud F.** Epidemiology and Diagnosis of *Helicobacter pylori* infection. *Helicobacter* 2015; **20** Suppl 1: 1-7 [PMID: 26372818 DOI: 10.1111/hel.12250]
- 20 **Ismail H, Morgan C, Griffiths P, Williams J, Jenkins G.** A Newly Developed Nested PCR Assay for the Detection of *Helicobacter*

- pylori* in the Oral Cavity. *J Clin Gastroenterol* 2016; **50**: 17-22 [PMID: 25811111 DOI: 10.1097/MCG.0000000000000310]
- 21 **Ogaya Y**, Nomura R, Watanabe Y, Nakano K. Detection of *Helicobacter pylori* DNA in inflamed dental pulp specimens from Japanese children and adolescents. *J Med Microbiol* 2015; **64**: 117-123 [PMID: 25332373 DOI: 10.1099/jmm.0.079491-0]
 - 22 **Puz S**, Innerhofer A, Ramharter M, Haefner M, Hirschl AM, Kovách Z, Rotter M, Makristathis A. A novel noninvasive genotyping method of *Helicobacter pylori* using stool specimens. *Gastroenterology* 2008; **135**: 1543-1551 [PMID: 18835389 DOI: 10.1053/j.gastro.2008.08.006]
 - 23 **Vécei A**, Innerhofer A, Binder C, Gizci H, Hammer K, Bruckdorfer A, Riedl S, Gadner H, Hirschl AM, Makristathis A. Stool polymerase chain reaction for *Helicobacter pylori* detection and clarithromycin susceptibility testing in children. *Clin Gastroenterol Hepatol* 2010; **8**: 309-312 [PMID: 20005978 DOI: 10.1016/j.cgh.2009.12.002]
 - 24 **Scaletsky IC**, Aranda KR, Garcia GT, Gonçalves ME, Cardoso SR, Iriya K, Silva NP. Application of real-time PCR stool assay for *Helicobacter pylori* detection and clarithromycin susceptibility testing in Brazilian children. *Helicobacter* 2011; **16**: 311-315 [PMID: 21762271 DOI: 10.1111/j.1523-5378.2011.00845.x]
 - 25 **Schabereiter-Gurtner C**, Hirschl AM, Dragosics B, Hufnagl P, Puz S, Kovách Z, Rotter M, Makristathis A. Novel real-time PCR assay for detection of *Helicobacter pylori* infection and simultaneous clarithromycin susceptibility testing of stool and biopsy specimens. *J Clin Microbiol* 2004; **42**: 4512-4518 [PMID: 15472302 DOI: 10.1128/JCM.42.10.4512-4518.2004]
 - 26 **Lottspeich C**, Schwarzer A, Panthel K, Koletzko S, Rüssmann H. Evaluation of the novel *Helicobacter pylori* ClariRes real-time PCR assay for detection and clarithromycin susceptibility testing of *H. pylori* in stool specimens from symptomatic children. *J Clin Microbiol* 2007; **45**: 1718-1722 [PMID: 17392440 DOI: 10.1128/JCM.00103-07]
 - 27 **Xiong LJ**, Tong Y, Wang Z, Mao M. Detection of clarithromycin-resistant *Helicobacter pylori* by stool PCR in children: a comprehensive review of literature. *Helicobacter* 2013; **18**: 89-101 [PMID: 23067446 DOI: 10.1111/hel.12016]
 - 28 **Noguchi N**, Rimbara E, Kato A, Tanaka A, Tokunaga K, Kawai T, Takahashi S, Sasatsu M. Detection of mixed clarithromycin-resistant and -susceptible *Helicobacter pylori* using nested PCR and direct sequencing of DNA extracted from faeces. *J Med Microbiol* 2007; **56**: 1174-1180 [PMID: 17761479 DOI: 10.1099/jmm.0.47302-0]

P- Reviewer: El-Zahaby SA, Malnick S

S- Editor: Qi Y **L- Editor:** A **E- Editor:** Wang CH





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: bpgoffice@wjgnet.com

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



ISSN 1007-9327



9 771007 932045