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Diagnostic accuracy of a real-time PCR assay for detection of *Helicobacter pylori* and resistance to clarithromycin and levofloxacin directly from stool

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ABSTRACT. – OBJECTIVE: The non-invasive detection of *Helicobacter pylori (H. pylori)* and its resistance to clarithromycin and levofloxacin significantly improves the management of infected patients by enabling tailored eradication treatments without the need for endoscopic procedures. This study aimed to assess the effectiveness of real-time PCR (RT-PCR) assays in identifying *H. pylori* infection and antibiotic resistance in stool and gastric biopsy specimens.

PATIENTS AND METHODS: Stool and gastric biopsy samples were collected from patients within three days of post-hospitalization. A total of 115 samples were analyzed for *H. pylori* infection, and an additional 115 samples were evaluated for resistance to clarithromycin and levofloxacin using an RT-PCR-based molecular test. Statistical analyses were performed using (SPSS 26.0 IBM Corp., Armonk, NY, USA).

RESULTS: Among 115 patients (53 males, average age 50.8±13.2 years), *H. pylori* was detected in 93.1% of stool samples and 93.9% of gastric biopsies. The RT-PCR assay demonstrated a sensitivity of 99.1% and a specificity of 100%, with an overall diagnostic accuracy of 99.1%. Clarithromycin resistance was found in 37.3% of stool and 46.9% of gastric biopsy specimens, with the assay showing 79.6% sensitivity and 98.4% specificity. Levofloxacin resistance was identified in 32.1% of stool samples and 31.3% of gastric biopsies, with 86.3% sensitivity and 91.1% specificity of the molecular test.

CONCLUSIONS: The RT-PCR-based detection of *H. pylori* and its resistance to clarithromycin and levofloxacin in stool samples represents a promising approach to enhance eradication therapy outcomes, potentially improving treatment efficacy.

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Key Words:
Helicobacter pylori, Antibiotic resistance, Stool, Molecular diagnosis, Real-time polymerase chain reaction.

Introduction

Helicobacter pylori (*H. pylori*), a spiral-shaped, Gram-negative bacterium, infects approximately 50% of humans worldwide[1,2]. Despite a declining incidence in developed countries, *H. pylori* infection remains a major cause of morbidity and mortality worldwide[3]. *H. pylori* infection is associated with a range of serious gastroduodenal diseases, including chronic gastritis, peptic ulcer disease, mucosa-associated lymphoid tissue lymphoma, and gastric cancer, underscoring the need for *H. pylori* eradication worldwide[4]. Given its carcinogenic potential, *H. pylori* has been classified as a class I carcinogen by the World Health Organization (WHO) in 1994 and the U.S. Department of Health and Human Services (HHS) in 2022[5,6]. Therefore, the eradication of *H. pylori* is considered to be very important for the management of these diseases.

The diagnosis of *H. pylori* infection involves both invasive and non-invasive methods[7]. Detection of *H. pylori* from stool samples is non-invasive and easy to perform in epidemiological studies and diagnosis of infection in children[8]. In recent years, several molecular approaches for the detection of *H. pylori* infection and its antimicrobial resistance have been developed[9]. Notably, the non-invasive molecular testing through real-time polymerase chain reaction (RT-PCR) on stool samples has shown high sensitivity and specificity[10]. A meta-analysis[11] has identified that the bacterial *23S* ribosomal RNA subunit gene as the most dependable marker for diagnosing *H. pylori* infection on stool samples, with a sensitivity and specificity of 82% and 99%, respectively. Therefore, there is an increasing need for non-invasive methods to diagnose *H. pylori* infection and antimicrobial resistance.

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The prevalence of *H. pylori* antibiotic resistance has been increasing remarkably worldwide, and the success rate of *H. pylori* eradication is decreasing[12]. The main cause of treatment failure has been reported as antimicrobial resistance; the resistance levels for clarithromycin and levofloxacin have reached 20%-50%, in China alone[13,14]. Although RT-PCR assay is the most sensitive and specific method for detecting *H. pylori* in gastric biopsy specimens, there is a lack of studies comparing the RT-PCR assay using different sample types[15]. Therefore, this study aims to evaluate the diagnostic accuracy of the stool-based *H. pylori* RT-PCR test as an alternative approach for detecting *H. pylori* infection and determining antibiotic resistance and compared to the gastric biopsy specimens *H. pylori* RT-PCR test, which serves as the reference standard.

Patients and Methods

Study Design and Assessment

This study was conducted between April 2023 and August 2023, patients were eligible if they were aged 18-75 years and had confirmed *H. pylori* infection *via* the C-urea breath test (UBT)[14]. A total of 115 patients from outpatient clinics met the eligibility criteria and were enrolled.

Inclusion Criteria: male or female patients aged 18-75 years; diagnosis of *H. pylori* infection by C-UBT test[14]; presence of upper gastrointestinal symptoms (e.g., epigastric pain, acid reflux, heartburn, epigastric distention, nausea); consent to participate *via* signed informed consent form. Exclusion Criteria: antibiotic, bismuth, or proton pump inhibitor (PPI) use within the last four weeks; recurrent or long-term use of macrolides and penicillin; serious primary diseases (liver, kidney, heart, brain, lung, endocrine system, hematopoietic system); significant liver or kidney insufficiency; pregnancy, lactation, positive pregnancy test, or intent to become pregnant within six months.

Stool and gastric biopsy samples were collected from patients 1 to 3 days post-hospitalization. The *H. pylori* infection and antibiotic resistance (clarithromycin and levofloxacin) were assessed using RT-PCR on both stool and gastric biopsy samples, employing the clarithromycin, quinolone-resistant and non-resistant *Helicobacter pylori* Nucleic Acid Amplification Test kit (Jiangsu Mole Bioscience Co., Ltd, Taizhou, Jiangsu, China).

Ethics Approval

This study followed the Consolidated Standards of Reporting Trials (CONSORT) guidelines. Written informed consent was obtained from all participants involved in the study. The study protocol was approved by the Institutional Ethics Board of the Civil Aviation General Hospital, Beijing, China (No. 2023-L-K-05). The trial was registered in the Chinese Clinical Trials Registration (www.chictr.org.cn) with the registration number ChiCTR2300070267.

Statistical Analysis

Statistical analyses were conducted using SPSS 26.0 (IBM Corp., Armonk, NY, USA). The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of each test were investigated. The agreement between stool and gastric biopsy sample PCR test was estimated with the kappa coefficient. A p-value lower than 0.05 was considered statistically significant.

Results

In this study, 115 patients were enrolled, comprising 53 males and 62 females, with an average age of 50.8±13.2 years. *H. pylori* infection was detected in 108 patients (93.1%) of stool samples and 109 patients (93.9%) through gastric biopsy samples *via* RT-PCR test. As shown in Table I, the RT-PCR assay achieved a sensitivity of 99.1% and a specificity of 100%, resulting in a diagnostic accuracy of 99.1%. The agreement between RT-PCR results from stool and gastric biopsy samples was excellent, evidenced by a Kappa score of 0.929 (p<0.001).

Table I. Performance of RT-PCR detection of *H. pylori* in stool and gastric biopsy samples.

	Sensitivity	Specificity	PPV	NPV	Accuracy	Kappa value	*p*-value
Analytical performance	99.1%	100%	100%	87.5%	99.1%	0.929	0.001

PPV: positive predictive value; NPV: negative predictive value; RT-PCR: real-time polymerase chain reaction.

Table II. Performance of RT-PCR detection of clarithromycin and levofloxacin resistance in stool and gastric biopsy samples.

Antibiotic resistance	Sensitivity	Specificity	PPV	NPV	Accuracy	Kappa value	*p*-value
Clarithromycin	79.6%	98.4%	97.7%	84.5%	78.0%	0.788	0.001
Levofloxacin	83.3%	91.1%	81.1%	92.3%	74.4%	0.739	0.001

PPV: positive predictive value; NPV: negative predictive value; RT-PCR: real-time polymerase chain reaction.

Clarithromycin resistance was identified in 43 patients (37.3%) *via* stool samples and 54 patients (46.9%) through gastric biopsy samples. In Table II, the sensitivity and specificity of the RT-PCR test for clarithromycin resistance were 79.6% and 98.4%, respectively, with an overall diagnostic accuracy of 78.0%. The concordance between the RT-PCR results for clarithromycin resistance from stool and gastric biopsy samples was good, with a Kappa value of 0.788 ($p<0.001$).

Levofloxacin resistance was detected in 37 patients (32.1%) from stool samples and 36 patients (31.3%) from gastric biopsy samples. As shown in Table II, the RT-PCR test for levofloxacin resistance exhibited a sensitivity of 86.3% and a specificity of 91.1%, with a diagnostic accuracy of 74.4%. The concordance for levofloxacin resistance between stool and gastric biopsy samples' RT-PCR test was also good, indicated by a Kappa score of 0.739 ($p<0.001$).

Discussion

H. pylori infection is the most popular chronic bacterial infection worldwide, frequently associated with a wide range of gastrointestinal and extraintestinal disorders[16]. The role of *H. pylori* in the pathogenesis of chronic atrophic gastritis, peptic ulcer disease, and functional dyspepsia, along with its links to various malignancies, autoimmune conditions, and other conditions, significantly influences our understanding and management approaches for these diseases[17-19]. This extensive impact underscores the need for precise diagnostic measures.

To accurately diagnose *H. pylori* infection, healthcare professionals have access to a range of both invasive and non-invasive techniques. However, no single test emerges as the definitive standard[20]. Invasive diagnostic procedures, such as histology, culture, and rapid urease tests, involve obtaining gastric biopsy specimens during gastroduodenoscopy, which, while effective, may carry risks and cause discomfort to patients[21,22]. Conversely, non-invasive diagnostic methods include urea breath tests, stool antigen tests, and serological assays[15]. Among these, recent advancements have highlighted the efficacy of RT-PCR in fecal samples, noted for its high sensitivity and specificity[23]. Specifically, the *23SrRNA* gene is utilized as a precise marker in RT-PCR-based fecal analyses for detecting *H. pylori* infection[24]. Our research confirmed this by identifying *H. pylori* in 93.1% of stool samples and 93.9% of gastric biopsy samples. The RT-PCR test demonstrated outstanding sensitivity (99.1%), specificity (100%), and diagnostic accuracy (99.1%), emphasizing its reliability. This is further supported by the strong concordance between the stool and gastric biopsy results (Kappa=0.929, $p<0.001$).

A critical aspect of managing *H. pylori* infection involves monitoring its antibiotic susceptibility, which is important to ensure the efficacy of treatment regimens. However, routine susceptibility testing is not commonly conducted due to its invasive nature, the limited availability of culture facilities, and cost considerations. Clarithromycin, a macrolide antibiotic, plays a pivotal role in *H. pylori* eradication by inhibiting protein synthesis[25]. Nonetheless, the increasing resistance to clarithromycin, primarily due to mutations in the *23SrRNA* ribosomal component, poses a significant challenge and frequently leads to treatment failures[26]. Empirical first-line treatments that incorporate clarithromycin have shown disappointing success rates, with only approximately 18% achieving an eradication threshold above 85%, while about 60% fail to reach even 80% effectiveness[27]. Resistance to clarithromycin, metronidazole, and levofloxacin is highly prevalent, complicating the development of effective treatment strategies[28,29]. Our findings indicate resistance rates of 37.3% for clarithromycin and 32.1% for levofloxacin in stool samples, figures that closely mirror those observed in gastric samples. The consistency between RT-PCR results from both types of samples validates the stool RT-PCR test as an effective method for assessing resistance to these antibiotics.

Conclusions

Routine culture and antimicrobial susceptibility testing are rarely conducted in clinical practice, highlighting the need for continuous research into *H. pylori* resistance to improve treatment methods. A novel molecular test utilizing RT-PCR technology has emerged as a critical tool in this effort. This non-invasive method provides an accurate diagnosis of *H. pylori* infection and identifies resistance to clarithromycin and levofloxacin. These capabilities are crucial for enhancing patient compliance, ensuring prompt and precise treatment interventions, improving the success rates of *H. pylori* eradication, and thereby reducing the risk of associated diseases.

Conflict of Interest
The authors declare that they have no conflict of interest.

Availability of Data and Materials
The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request. The datasets are also available in the Chinese Clinical Trial Registry.

Ethics Approval
The study was conducted in accordance with the Helsinki Declaration and has been approved by the Institutional Ethics Board of the Civil Aviation General Hospital (registration number 2023-L-K-05). This study followed the Consolidated Standards of Reporting Trials (CONSORT) guidelines.

Clinical Trial Registration
The trial was registered in the Chinese Clinical Trials Registration (available at: www.chictr.org.cn) with the registration number ChiCTR2300070267.

Informed Consent
We obtained informed consent from patients or their immediate family members.

Authors' Contributions
CJP: study design, data analysis, data interpretation, and manuscript writing. BQX: study design and critical revision of the manuscript. LZ, ZLL, WH, ZXL, XDL, CY, and DHO: material preparation, data collection. FCJ and HK: patient recruitment, study coordination, and study supervision. All authors have read and approved the submitted manuscript.

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