Microb Health Dis 2023; 5: e956

EVALUATION OF REAL-TIME PCR COMPARED TO CULTURE FOR THE DETECTION OF CLARITHROMYCIN RESISTANT *HELICOBACTER PYLORI* IN THE IRISH HEALTHCARE SETTING

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Abstract – *Objective:* Molecular methods offer a more rapid alternative for the detection of *H. pylori* resistance to antibiotics than traditional culture-based methods. The aim of the study was to evaluate the RIDAGENE *H. pylori* real-time PCR assay compared to culture-based methods for the detection of *H. pylori* and clarithromycin resistance using gastric biopsies.

Patients and Methods: Following ethical approval and informed consent, adults were recruited prospectively from Tallaght University Hospital and Letterkenny University Hospital, Ireland, regardless of *H. pylori* treatment history. During routine gastroscopy, subjects had 1 antrum and 1 corpus biopsy taken for *H. pylori* culture and DNA extraction. Clarithromycin susceptibility testing on cultures was performed by ETEST (Biomerieux, UK). The RIDAGENE *H. pylori* assay (R-Biopharm AG, Germany) was used for detection of *H. pylori* DNA and clarithromycin resistance-associated point mutations.

Results: In all, samples from 191 culture-positive patients (mean age 48.4 ± 15.3 years; 45.0% (N=86) female) were analysed. The RIDAGENE assay detected *H. pylori* in 100% of biopsy samples from which *H. pylori* was cultured. The clarithromycin resistance rate by culture was significantly higher than by real-time PCR (49.2% (N=94/191) and 38.7% (N=74/191, respectively; p=0.04; χ^2 test). Results agreed between both methods in 84.3% (N=161/191) of cases. The sensitivity and specificity of the RIDAGENE assay compared to culture for the detection of clarithromycin resistance were 74.0% (95% CI: 64.0-82.4%) and 94.7% (95% CI: 88.1-98.3%), respectively. The positive predictive value was 93.4% (95% CI: 85.7-97.1%) and the negative predictive value was 78.3% (95% CI: 71.9-83.5%).

Conclusions: The RIDAGENE assay detected *H. pylori* in all culture-positive samples. However, the low sensitivity compared to culture for clarithromycin susceptibility testing in our cohort may limit its use to cases where culture-based methods are unsuccessful. Further studies are required to fully characterise *H. pylori* clarithromycin resistance mechanisms in our study population.

Keywords: *Helicobacter pylori*, Clarithromycin, Antimicrobial resistance, Antimicrobial susceptibility testing, Molecular methods, Real-time PCR.

INTRODUCTION

Helicobacter pylori (*H. pylori*) causes one of the world's most prevalent bacterial infections affecting over half of the global population[1]. Classified as a 'Class 1' carcinogen by the World Health Organisation means that effective treatment and eradication of this bacterium is imperative in preventing the progression of gastritis and the development of gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma[2]. Typical treatment for *H. pylori* infection includes the use of proton-pump inhibitors (PPIs) with two or three antimicrobial drugs. Globally, *H. pylori* drug resistance is increasing, leading to treatment failures[3]. As a result, consensus guidelines recommend antimicrobial resistance surveillance so that the local prevalence of antimicrobial resistance can be considered when choosing an appropriate anti-*H. pylori* regimen for each population[4-9]. Moreover, clarithromycin antimicrobial susceptibility testing (AST) is now recommended prior to prescribing any clarithromycin-containing therapy[4].

AST can be performed on gastric biopsies using culture-based methods or by molecular methods involving DNA isolation followed by the polymerase chain reaction (PCR), DNA hybridisation or DNA sequencing. *H. pylori* is generally considered difficult to culture as it is microaerophilic, highly fastidious and slow to grow (up to 7 days)[10]. This means that many laboratories don't routinely culture the bacterium for AST. PCR can be used to analyse previously identified genes that have been linked to antibiotic resistance and represents a faster approach than *H. pylori* culture and AST. Moreover, molecular methods for the detection of *H. pylori* are less impacted by low bacterial load, which can often result due to PPI use. Single point mutations within the gene encoding the 23S ribosomal RNA (rRNA) component result in clarithromycin resistance in *H. pylori* by altering the conformation of the drug target[11]. The RIDAGENE *H. pylori* kit (R-Biopharm, Germany) is a multiplex real-time PCR assay that detects (i) *H. pylori* by targeting the 16S rRNA gene and (ii) clarithromycin resistance by targeting the three most common resistance-associated mutations in the 23S rRNA gene (A2146C, A2146G and A2147G). As *H. pylori* exhibits a diverse genomic structure with a high mutation rate[12], PCR assays should be validated locally prior to recommendation for clinical diagnostics. The aim of this study was to evaluate the RIDAGENE *H. pylori* assay compared to culture for the detection of *H. pylori* and clarithromycin resistance using gastric biopsies in an Irish context.

PATIENTS AND METHODS

Ethics Statement

The study was approved by the Joint Research Ethics Committee of Tallaght University Hospital and St. James's Hospital [Reference: REC-2020-03- List 9 – Amendment (18); approved 23/03/2020] and the Research Ethics Committee of Letterkenny University Hospital [Reference: *Helicobacter pylori* antibiotic resistance; approved 16/08/2021]. The procedures followed were in accordance with the Ethical Standards of the Helsinki Declaration of 1975, as revised in 2000.

Study Population and Sample Collection

Patients attending Tallaght University Hospital, Dublin, Ireland and Letterkenny University Hospital, Donegal, Ireland for upper gastrointestinal endoscopy were invited to partake in the study, regardless of *H. pylori* treatment history. Inclusion criteria were (1) ability and willingness to participate in the study and to provide informed consent; and (2) confirmed *H. pylori* infection by culture. Exclusion criteria were (1) age less than 18 years; (2) pregnancy or lactation; (3) severe intercurrent illness; (4) recent antimicrobial use (within 4 weeks); and (5) bleeding problems or use of blood thinning drugs. Following receipt of informed consent, one antrum and one corpus biopsy were taken from each patient and placed directly into DENT's transport medium [brain heart infusion broth containing 2.5% (w/v) yeast extract, 5% sterile horse serum and *H. pylori* Selective Supplement (Oxoid, United Kingdom (UK)] for transport to the research laboratory.

H. pylori Culture

The corpus and antrum biopsies from each patient were spread onto a Columbia blood agar plate containing 5% laked horse blood and incubated at 37°C in a microaerobic atmosphere using the CampyGen system (Oxoid Ltd., UK). The biopsy samples were then stored at -20°C until processed for DNA extraction. Inoculated plates were examined for the presence of *H. pylori* for up to 7 days. *H. pylori* was identified by visual inspection of the colonies, a positive urease test and by PCR. *H. pylori* colonies were dispersed in maximum recovery diluent (Oxoid Ltd) until the turbidity was that of a 3 McFarland standard. A swab was dipped into this solution and spread evenly onto Columbia blood agar containing 5% laked horse blood, before a clarithromycin ETEST (Biomerieux) strip was added. The plate was incubated for 48-72 hours at 37° under microaerobic conditions. AST results were interpreted according to the guidelines and criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST, Clinical Breakpoint Tables Version 13.1, valid from 29th June 2023)[13]. Strains with a minimum inhibitory concentration (MIC) >0.25 mg/L were considered resistant to clarithromycin.

DNA Extraction and PCR

DNA was extracted from the biopsies of culture-positive patients using the QIAmp DNA Mini Kit (QIAGEN, UK). 5 μL of the extracted DNA was then used in the RIDAGENE *Helicobacter pylori* assay (R-Biopharm AG, Germany) for the detection of *H. pylori* and clarithromycin resistance-associated point mutations. The PCR was performed on the QuantStudio 5 (Applied Biosciences, UK) using settings recommended in the product insert of the RIDAGENE *Helicobacter pylori* assay.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 9.5.1 (La Jolla, CA, USA). Continuous variables are presented as arithmetic mean and standard deviation. Categorical variables are presented as percentages and were compared using the two-tailed X^2 test. A p-value less than 0.05 was considered significant.

RESULTS

Patient Demographics

In all, samples from 191 culture-positive patients (mean age 48.4 ± 15.3 years) were included in the study (Table 1). 86 (45.0%) samples were from females and 105 (55.0%) were from males.

TABLE 1. DEMOGRAPHICS OF INCLUDED PATIENTS.

Demographic	N = 191
Mean Age (years)	48.4
Standard deviation	15.3
Age range	19-83
Male	105 (55.0%)
Female	86 (45.0%)

Clarithromycin Resistance Rates

A normal distribution of clarithromycin MICs obtained by culture and ETEST was observed across the strains, with a clear separation between resistant and susceptible strains (Figure 1). The RIDAGENE assay detected *H. pylori* in 100% (N=191) of biopsy samples from which *H. pylori* was cultured (Table 2). Culture-based methods detected clarithromycin resistance in a significantly higher percentage of cases than molecular methods (49.2% *vs.* 38.7%, respectively; p=0.04, X^2=4.3) (Table 2). Antimicrobial susceptibility results agreed between both methods in 84.3% (N=161/191) of cases (Table 2).

Diagnostic Accuracy

The diagnostic accuracy statistics of the RIDAGENE assay compared to culture and ETEST for the detection of clarithromycin resistance are shown in Table 3. The sensitivity and specificity of the RIDAGENE assay compared to culture were 74.0% and 94.7%, respectively. The positive predictive value (PPV) was 93.4% and the negative predictive value (NPV) was 78.3% (Table 3).

DISCUSSION

While culture-based methods are considered the gold standard for *H. pylori* AST, molecular methods represent a more rapid approach than *H. pylori* culture. Molecular methods can be performed directly on DNA isolated from biopsy material, eliminating the need for culture which is often unsuccessful. In this study, the RIDAGENE assay detected *H. pylori* DNA in all 191 (100%) samples from which *H. pylori* was cultured. Although we did not evaluate the diagnostic accuracy statistics of the RIDAGENE assay for detecting *H. pylori* by analysing culture nega-

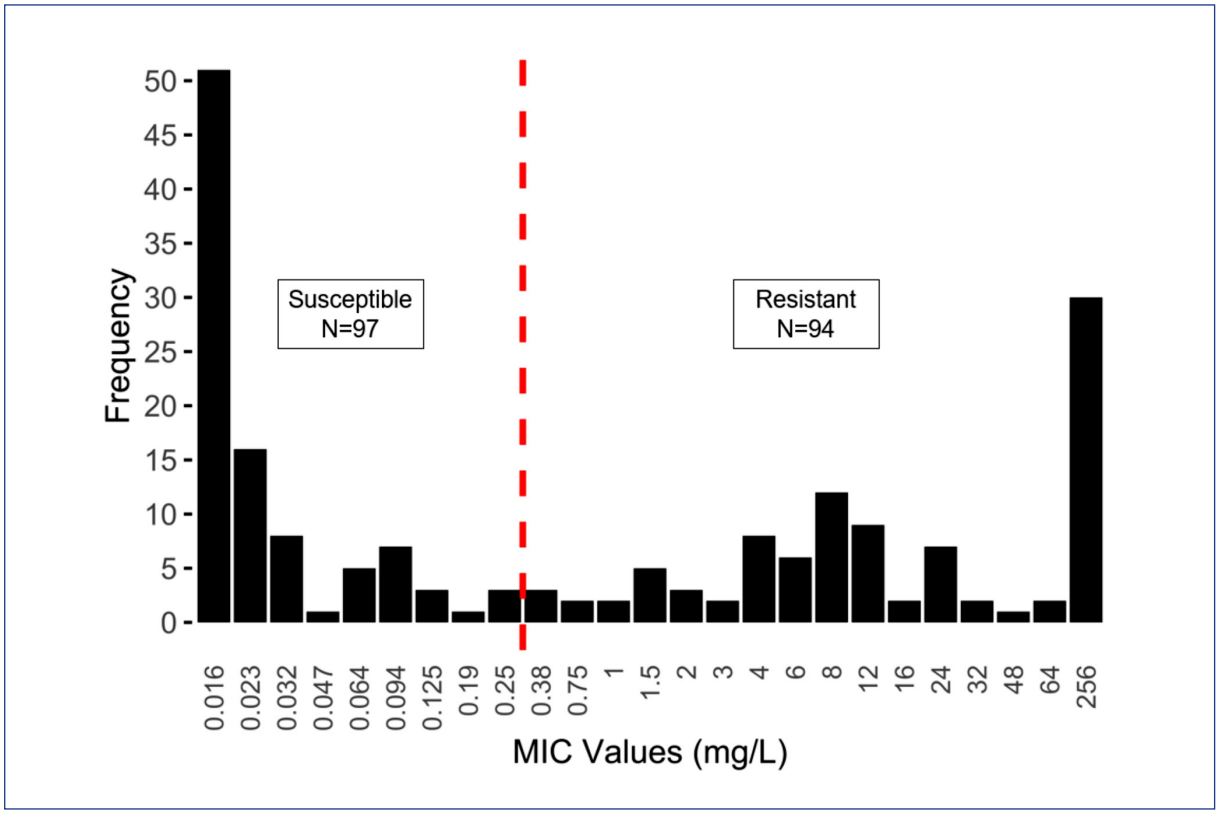

Figure 1. Distribution of MICs for clarithromycin obtained by H. pylori culture and ETEST.

TABLE 2. AGREEMENT BETWEEN CULTURE-BASED METHODS AND THE RIDAGENE ASSAY FOR THE DETECTION OF *H. PYLORI* AND CLARITHROMYCIN RESISTANCE.

	Culture		RIDAGENE assay		Agreement*	
	N	%	N	%	N	%
H. pylori	191	100	191	100	191	100
Susceptible	97	50.8	117	61.3	161	84.3
Resistant	94	49.2	74	38.7		

*The number of cases in which the results from both tests were the same.

TABLE 3. THE DIAGNOSTIC ACCURACY OF THE RIDAGENE ASSAY COMPARED TO CULTURE AND ETEST OR THE DETECTION OF CLARITHROMYCIN RESISTANCE.

Statistic	Value	95% CI
Sensitivity	74.0%	64.0-82.4%
Specificity	94.7%	88.1-98.3%
Positive Likelihood ratio	14.1%	5.9-33.3%
Negative likelihood ratio	0.3%	0.2-0.4 %
Positive predictive value	93.4%	85.7-97.1%
Negative predictive value	78.3%	71.9-83.5%
Accuracy	84.3%	78.3-89.1%

tive samples, other have shown excellent sensitivity and specificity of the RIDAGENE assay compared to culture in this regard[14,15]. Taken together, these findings support the use of the RIDAGENE assay for diagnosing *H. pylori* in gastric biopsy samples.

In terms of clarithromycin AST, results were in agreement between culture and the RIDAGENE assay in 84.3% (N= 161/191) of cases. However, a significantly higher overall rate of clarithromycin resistance was detected by culture compared to the RIDAGENE assay (49.2% *vs.* 38.7%, respectively; p=0.04). This is reflected in the sub-optimal sensitivity and NPV of the assay compared to culture (74.0% and 78.3%, respectively). Previous studies have demonstrated better diagnostic accuracy of the RIDAGENE assay in the past. Bénéjat et al[16] reported that the sensitivity was 97.14%, the specificity was 100%, the PPV was 100%, and the NPV was 98.48% for the detection of clarithromycin resistance using the RIDAGENE assay on the BD-MAX system (Becton Dickinson, France). Sensitivity, specificity, PPV and NPV were all 100% when the RIDAGENE assay was evaluated using the Elite Ingenius system (Elitech, France) by the same group[17]. However, the diagnostic accuracy of the RIDAGENE assay in these investigations was compared to in-house PCR evaluating the same DNA mutations[16,17], rather than the phenotypic culture-based AST results analysed in the current study. One other study from Belgium compared the RIDAGENE assay to culture for the detection of clarithromycin resistance, albeit with a smaller sample size than our study (N=42) and found its sensitivity and specificity to be 92% and 97%, respectively[18]. Differences in the sensitivity of the assay between our study and the Belgian study may be due to genetic diversity between the infecting strains in the different populations. The RIDAGENE assay is limited to the detection of the 3 most common clarithromycin resistance-associated DNA mutations in the 23S rRNA gene (A2146C, A2146G and A2147G). These point mutations are thought to account for approxi-

mately 90% of clarithromycin resistance[19]. However, the assay does not detect less common mutations which have also been shown to confer clarithromycin resistance[20-23]. In addition to point mutations in genes encoding drug targets, other resistance mechanisms, such as efflux pumps and biofilm formation, have been reported to contribute to clarithromycin resistance in *H. pylori*[24-27]. Further studies are required to investigate whether alternative mutations and/or efflux pumps and biofilm formation contribute to clarithromycin resistance in *H. pylori* strains infecting our patient cohort.

CONCLUSIONS

The RIDAGENE assay detected *H. pylori* in all biopsy tissue samples that were culture positive. However, the lower sensitivity and NPV of the assay compared to culture for the detection of clarithromycin resistance in our cohort may limit its use for AST. Further studies are required to fully characterise the clarithromycin resistance mechanisms in *H. pylori* strains circulating in our patient cohort.

Conflict of Interest

The authors have no conflicts of interest to declare.

Acknowledgments

Not applicable.

Informed Consent

Patients were recruited only if they had the ability and willingness to provide informed consent. Only after receipt of informed consent were samples collected and demographic data recorded.

Ethics Statement

The study was approved by the Joint Research Ethics Committee of Tallaght University Hospital and St. James's Hospital [Reference: REC-2020-03- List 9 – Amendment (18); approved 23/03/2020] and the Research Ethics Committee of Letterkenny University Hospital [Reference: *Helicobacter pylori* antibiotic resistance; approved 16/08/2021]. The procedures followed were in accordance with the Ethical Standards of the Helsinki Declaration of 1975, as revised in 2000.

Funding

This work was supported by the Health Research Board [HRB-2019-APA-2019-030 and HRB-ILP-2022-048 to SMS].

Author Contributions

SDM, TJB, IM, AK and SMS performed experiments. SDM, TJB and SMS analysed data. SDM and SMS wrote the manuscript. VP, KVDM and DMN recruited patients and collected samples. All authors critically revised the manuscript and provided final approval before submission for publication.

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Data Availability Statement

All data generated or analysed during this study are included in this published article.

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