**Name of Journal: *World Journal of Gastroenterology***

**Manuscript NO: 37541**

**Manuscript Type: ORIGINAL ARTICLE**

***Basic study***

**Can bacterial virulence factors predict antibiotic resistant *Helicobacter pylori* infection?**

Brennan DE *et al. H. pylori* virulence factors

**Denise E Brennan, Colin Dowd, Colm O’Morain, Deirdre McNamara,Sinéad M Smith**

**Denise E Brennan, Colin Dowd, Colm O’Morain, Deirdre McNamara,Sinéad M Smith,** Department of Clinical Medicine, School of Medicine, Trinity College Dublin, Dublin D24, Ireland

**ORCID number:** Denise E Brennan ([0000-0001-8200-3181](http://orcid.org/0000-0001-8200-3181)); Colin Dowd ([0000-0003-0608-0585](http://orcid.org/0000-0003-0608-0585)); Colm O’Morain ([0000-0002-1847-6782](http://orcid.org/0000-0002-1847-6782)); Deirdre McNamara (0000-0003-2324-3382);Sinéad M Smith ([0000-0003-3460-3590](http://orcid.org/0000-0003-3460-3590)).

**Author contributions:** McNamara D and Smith SM contributed equally to the study; McNamara D and Smith SM designed the study and coordinated the research; Brennan DE, Dowd C and Smith SM performed experiments and analysed data; O’Morain C and McNamara D recruited patients; Brennan DE, McNamara D and Smith SM wrote the paper; all authors provided critical input into the final manuscript.

**Supported by** the Health Research Board (HRA-POR-2014-526).

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

**Conflict-of-interest statement:** None to declare.

**Data sharing statement:** There is no additional data to share.

**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: Sinead Smith, PhD, Ussher Assistant Professor in Applied and Translational Medicine,** Rm 1.46, Department of Clinical Medicine, Trinity Centre, Adelaide and Meath Hospital, Tallaght, Dublin D24, Ireland. smithsi@tcd.ie

**Telephone:** +353-1-8963844

**Fax:** +353-1-8962988

**Received:** January 10, 2018

**Peer-review started:** January 10, 2018

**First decision:** January 18, 2018

**Revised:** February 5, 2018

**Accepted:**

**Article in press:**

**Published online:**

## Abstract

***AIM***

To evaluate the association between virulence factor status and antibiotic resistance in *H. pylori*-infected patients in Ireland.

***METHODS***

DNA was extracted from antral and corpus biopsies obtained from 165 *H. pylori*-infected patients. Genotyping for clarithromycin and fluoroquinolone-mediating mutations was performed using the Genotype HelicoDR assay. *CagA* and *vacA* genotypes were investigated using PCR.

***RESULTS***

Primary, secondary and overall resistance rates for clarithromycin were 50.5% (*n* = 53/105), 78.3% (*n* = 47/60) and 60.6% (*n* = 100/165), respectively. Primary, secondary and overall resistance rates for fluoroquinolones were 15.2% (*n* = 16/105) and 28.3% (*n* = 17/60) and 20% (*n* = 33/165), respectively. Resistance to both antibiotics was 12.4% (*n* = 13/105) in treatment-naïve patients, 25% (*n* = 15/60) in those previously treated and 17% (*n* = 28/165) overall. A *cagA*-positive genotype was detected in 22.4% (*n* = 37/165) of patient samples. The dominant *vacA* genotype was S1/M2 at 44.8% (*n* = 74/165), followed by S2/M2 at 26.7% (*n* = 44/165), S1/M1 at 23.6% (*n* = 39/165) and S2/M1 at 4.8% (*n* = 8/165). Primary clarithromycin resistance was significantly lower in *cagA*-positive strains than in *cagA*-negative strains [32% (*n* = 8/25) *vs* 56.3% (*n* = 45/80); *P* = 0.03]. Similarly, in patients infected with more virulent *H. pylori* strains bearing the *vacA* s1 genotype, primary clarithromycin resistance was significantly lower than in those infected with less virulent strains bearing the *vacA* s2 genotype, [41% (*n* = 32/78) *vs* 77.8% (*n* = 21/27); *P* = 0.0001]. No statistically significant association was found between primary fluoroquinolone resistance and virulence factor status.

***CONCLUSION***

Genotypic *H. pylori* clarithromycin resistance is high and *cagA*-negative strains are dominant in our population. Less virulent (*cagA*-negative and *vacA* S2-containing) strains of *H. pylori* are associated with primary clarithromycin resistance.

## Key words: *Helicobacter pylori*; Antibiotic resistance; Virulence factor; Clarithromycin; Fluoroquinolone; VacA; CagA

**© The Author(s) 2018.** Published by Baishideng Publishing Group Inc. All rights reserved.

## Core tip: The management of *H. pylori* infection is challenging, largely due to the emergence of antibiotic resistance. A greater understanding of local antibiotic resistance rates is important in determining the most appropriate treatment regimen in a given population. Furthermore, insight into the virulence of the infecting strains and the association between virulence and antibiotic resistance could potentially be an avenue to explore in the effort to improve eradication rates. This study provides and update on the prevalence of clarithromycin and fluoroquinolone resistance in Ireland and demonstrates that less virulent strains of *H. pylori* are predictive of primary clarithromycin resistance.

Brennan DE, Dowd C, O’Morain C, McNamara D,Smith SM.Can bacterial virulence factors predict antibiotic resistant *Helicobacter pylori* infection?*World J Gastroenterol* 2018; In press

## INTRODUCTION

*Helicobacter pylori* infection causes acute and chronic gastritis, gastric and duodenal ulcers, and in rare cases gastric adenocarcinoma and MALT (mucosa-associated lymphoid tissue) lymphoma[1]. While its prevalence in the developed world has generally decreased, it is still high in indigenous populations and the developing world[2]. The Maastricht consensus recommends that all symptomatic *H. pylori*-infected adults are treated[1]. There are many different treatment options available, however the most common treatment for first-line eradication of *H. pylori* is triple therapy, which consists of two antibiotics (clarithromycin and amoxicillin) and a proton pump inhibitor, taken for 7-14 d. An efficacious therapy for *H. pylori* eradication is one that achieves an eradication rate of over 80%[1]. However, in many countries, the eradication rate for standard triple therapy has fallen below 80%. Indeed in a recent study in Ireland, the eradication rates of standard seven-day triple therapy were just 56.8% and 61% by intention-to-treat and per-protocol analysis, respectively[3]. There are several reasons that impact the efficacy of treatment for *H. pylori*; high bacterial load, high gastric acidity and poor patient compliance. However, undoubtedly the most important is the rapid emergence of antimicrobial resistant strains of *H. pylori*, particularly to clarithromycin[4-6]. Resistance to clarithromycin can decrease the success rate of clarithromycin-based triple therapy by up to 70%[7]. One study found that the presence of clarithromycin resistant strains in a patient infected with *H. pylori* predicted treatment failure almost perfectly[8].

*H. pylori* is a highly heterogeneous bacterium and its virulence varies geographically. Virulence factors not only contribute to the pathogenicity of the bacteria but may play a role in determining treatment outcome[9]. The most commonly studied virulence factors in *H. pylori* are encoded by the cytotoxin associated gene A (*cagA*) and the vacuolating associated gene A (*vacA*). There are at least 4 variable regions in the *vacA* gene; in the signal (s) region, of which one of two alleles can be present: s1 or s2, and in the middle (m) region, of which one of two alleles can be present; m1 or m2[10]. These variable regions display different levels of toxicity to host cells, with *vacA* s1/m1 being most cytotoxic, followed by s1/m2. The s2/m2 genotype has been found to induce little or no toxicity[11]. A possible relationship between virulence factors and antimicrobial resistance has been suggested. A study conducted in 2009 in Ireland reported that the absence of *cagA* may be a risk factor for developing metronidazole resistance[12]. This study aimed to provide an update on the prevalence of virulence factor genotypes and antibiotic resistance in Irish *H. pylori* strains and assess the relationship between clarithromycin and fluoroquinolone resistance with virulence factor status.

## 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 June 2017. 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 as indicated by a positive rapid urease test (TRI-MED Distributors, PTY LTD, Washington, United States) at 30 min and by 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 weeks) and (5) bleeding problems or use of blood thinning drugs.

### *Sample collection*

A single corpus and antrum biopsy from each patient were placed into 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. All isolated DNA was stored -20 °C until genotyping was performed.

### *Antimicrobial susceptibility genotyping*

Genotyping for clarithromycin and fluoroquinolone-mediating mutations was performed using the Genotype HelicoDR assay (Hain Lifescience GmbH, Nehren, Germany) according to the manufacturer’s instructions. Briefly, multiplex amplification of DNA regions of interest was performed using 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[13].

### *Virulence factor genotyping*

To determine virulence factor genotype, PCR was performed as previously described by Taneike *et al*[12] using the primers described in Table 1. C*agA* and *vacA* genotypes were evaluated by performing gel electrophoresis on the PCR products using 1% agarose gel.

***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. *P* values for categorical variables were calculated using the Fisher’s exact test/Pearson *χ2*-test. In all cases, a *P* value less than 0.05 was considered significant.

## RESULTS

### *Prevalence of genotypic antimicrobial resistance*

Samples from a total of 165 *H. pylori*-infected patients were analysed in the study. Patient demographics and clinical characteristics are shown in Table 2. 63.6% (*n* = 105) of patients had not been treated for *H. pylori* infection previously, while 36.4% (*n* = 60) had undergone at least one eradication treatment regimen (Table 2).

Primary resistance rates to clarithromycin and fluoroquinolones were 50.5% (*n* = 53/105; Table 3) and 15.2% (*n* = 16/105; Table 4), respectively. In those previously treated for *H. pylori* infection, the resistance rates for both clarithromycin and fluoroquinolones were higher at 78.3% (*n* = 47/60; Table 3) and 28.3% (*n* = 17/60; Table 4), respectively. Overall resistance rates, regardless of treatment history, were 60.6% (*n* = 100/165; Table 3) and 20% (*n* = 33/165; Table 4) for clarithromycin and fluoroquinolones, respectively. Among patients infected with a clarithromycin-resistant strain, the most common point mutation was A2147G, at 78% (*n* = 78/100; Table 3). The most common point mutation conferring resistance to fluoroquinolones in resistant patients was *gyr*91 D91Y, at 54.5% (*n* = 18/33; Table 4).

Dual resistance rates for clarithromycin and fluoroquinolones were 12.4% (*n* = 13/105) in the treatment naïve, 25% (*n* = 15/60) in those previously treated and 17% (*n* = 28/165) in all patients included (Table 5). The overall rate of dual susceptibility among the patients was 36.4% (*n* = 60/165; Table 5). Dual susceptibility was significantly higher in treatment-naïve patients versus those previously treated (46.6%, *n* = 49/105 versus 18.3%, *n* = 11/60; *P* < 0.05; Fisher’s exact test**).**

### *Distribution of H. pylori virulence-factor genotype*

Table 6 illustrates the distribution of *H. pylori* virulence factor genotype in infected patients. Overall, 22.4% (*n* = 37/165) of patients were infected with strains that were *cagA* positive and 77.6% (*n* = 128/165) that were *cagA* negative. The most prevalent *vacA* allele was S1/M2 at 44.8% (*n* = 74/165), followed by S2/M2, S1/M1 and S2/M1 at 26.7% (*n* = 44/165), 23.6% (*n* = 39/165) and 4.8% (*n* = 8/165), respectively (Table 6). Interestingly, the frequency of the *vacA* S1 genotype (the more virulent S region genotype) was significantly lower in those previously treated than the treatment-naïve group [58.3% (*n* = 35/60) *vs* 74.3% (*n* = 78/105) respectively; *P* < 0.05;Fisher’s exact test]. Additionally, the frequency of the S2/M2 genotype (the least virulent genotype) was significantly higher in those patients who have been treated previously [36.7% (*n* = 22/60) *vs* 21% (*n* = 22/105) respectively; *P* < 0.05; Fisher’s exact test; Table 6].

### *Less virulent strains of H. pylori are associated with primary clarithromycin resistance*

Next, the relationship between antibiotic resistance and virulence factor genotype was assessed. Analysis of all recruited patients revealed that genotypic resistance to clarithromycin was significantly lower in *cagA*-positive strains than in *cagA*-negative strains [40.5% (*n* = 15/37) *vs* 66.4% (*n* = 85/128); *χ2*= 8.04; *P* = 0.004; Pearson *χ2*test; Figure 1A]. When patients were sub-grouped into treatment-naïve (Figure 1B) and those previously treated (Figure 1C), clarithromycin resistance was also lower in *cagA*-positive strains compared to *cagA*-negative strains, although this only reached statistical significance in the treatment-naïve cohort [32% (*n* = 8/25) *vs* 56.3% (*n* = 45/80); *χ2* = 4.5; *P* = 0.03; Pearson *χ2* test; Figure 1B]. Similarly, in patients infected with more virulent *H. pylori* strains bearing the *vacA* s1 genotype, clarithromycin resistance was significantly lower than in those infected with less virulent strains bearing the *vacA* s2 genotype, when all patients were included [52.2% (*n* = 59/113) *vs* 78.8% (*n* = 41/52); *χ2* = 10.6; *P* = 0.001;Pearson *χ2* test; Figure 2A] and in those that were treatment-naïve [41% (*n* = 32/78) *vs* 77.8% (*n* = 21/27); *χ2* = 10.8; *P* = 0.0001; Pearson *χ2* test; Figure 2B], but not in patients that were previously treated (Figure 2C).

The frequency of resistance to fluoroquinolones in each virulence factor genotype was also examined. *CagA* status was not significantly associated with fluoroquinolone resistance when all patients were analysed (Figure 3A) or when the patients were sub-divided into those with primary infections (Figure 3B) and those previously treated (Figure 3C). While there was a significant association between the less virulent *vacA* s2 genotype and fluoroquinolone resistance when all patients were included [15% (*n* = 17/113) *vs* 30.8% (*n* = 16/52); *χ2* = 5.5; *P* = 0.02; Pearson *χ2* test; Figure 4A], this did not reach statistical significance in treatment naïve patients (Figure 4B) or those previously treated (Figure 4C).

Taken together, these findings indicate that the absence of *cagA* and the less virulent *vacA* genotypes (S2/M1 and S2/M2) may be predictors of primary clarithromycin resistance in treatment-naïve patients.

## DISCUSSION

This study aimed to provide an update on the prevalence of antibiotic resistance and distribution of virulence factor genotypes in *H. pylori* strains in Ireland. In addition we investigated whether virulence factor genotypes are associated with antibiotic susceptibility. Primary clarithromycin resistance among our patients was high at 50.5% and even higher in those previously treated at 78%. Among patients infected with a resistant strain, the most common point mutation conferring clarithromycin resistance was A2147G, in keeping with other studies[14-19]. Our primary clarithromycin resistance rate is high compared to rates reported in Europe, Asia Pacific and other countries[5,19-21]. Variations in *H. pylori* antibiotic resistance rates among different populations are influenced by previous antibiotic use, with studies demonstrating that previous exposure to macrolides increases the risk of clarithromycin resistant *H. pylori* infection[5,22]. The sharp increase in primary clarithromycin resistance from 3.9% in 1997 to 9.3% in 2008[23], to the current rate of 50.5% in 2017 is a cause for concern and is reflected in the poor eradication rate (56.8% ITT) for 7 days clarithromycin-based triple therapy recently reported from our centre[3]. In an effort to address increasing antibiotic resistance and falling eradication rates, the Irish *H. pylori* Working Group have recently highlighted the need for more widespread antibiotic resistance surveillance and extended *H. pylori* treatment durations[24]. It should be noted that antibiotic resistance was determined at the genetic level in the current study compared to culture and Etests in the earlier Irish surveys.

The primary and secondary rates of fluoroquinolone resistance were 15.2% and 28.3%, respectively. The primary rate of levofloxacin resistance has only risen slightly since the last Irish survey in 2008-2009, which reported a rate of 12%[25], and is in keeping with the 14.1% rate reported in Europe[5]. The most common point mutation conferring resistance to fluoroquinolones in our patients was *gyr91* D91Y. This contrasts with other studies in which *gyr91* D91N and *gyr87* N87K mutations were reported with highest frequency[14,16-18].

In our cohort, the overall frequency of *H. pylori* infections with strains containing the *cagA* gene was 22.4%. This has decreased since the distribution of the *cagA* genotype was last investigated in Ireland in 2009, with a frequency of 68% reported[12]. It is also lower than distributions reported in Cuba and Iran[26,27]. There is a well-known association between *cagA*-positive strains of *H. pylori* and peptic ulcer disease[28,29]. This relatively low frequency of *cagA*-positive genotype is not surprising given that the prevalence of peptic ulcer disease was also low in our cohort at 12.7% (Table 2), which is a decrease on the prevalence of peptic ulcer disease reported in the previous Irish study (17%[12]). The most prevalent *vacA* genotype in our cohort was S1/M2, followed by S2/M2, S1/M1 and S2/M1. This pattern is similar to the pattern reported in Ireland in 2009 as well as the studies mentioned above[12,26,27].

Interestingly, the frequency of the more virulent S1 genotype was significantly lower in those previously treated than the treatment-naïve group (58.3% *vs* 74.3%). Additionally, the frequency of the least virulent S2/M2 genotype was significantly higher in those previously treated previously (36.7% *vs* 21%). This is in accordance with a hypothesis described previously which suggests that more virulent strains elicit a stronger inflammatory response, enabling increased blood flow to the site of infection, therefore enhancing delivery of antibiotics and the potential for successful eradication[30]. Another potential explanation is that a more virulent strain of *H. pylori* may replicate faster and is therefore more susceptible to antibiotics such as clarithromycin and fluoroquinolones, whose mechanism of actions are to inhibit bacterial replication[31].

We found an inverse relationship between the virulence of the infecting strain and the presence of clarithromycin resistance: the absence of *cagA,* and the less virulent *vacA* genotypes (S2/M1 and S2/M2), may be indicators of clarithromycin resistance, in particular in treatment-naïve patients. The association between virulence factors and antibiotic resistance in *H. pylori* has been evaluated in other studies, with controversial results. Absence of *cagA* was found to be a risk factor for metronidazole resistance[12] and other studies have found an association between clarithromycin resistance mutations and the less virulent *vacA* genotypes[32,33]. Another report revealed that *cagE* and *vacA* S1 correlated with clarithromycin and metronidazole resistance[34], while others found that neither *cagA* nor *vacA* was associated with resistance[29,35-37]. There may be no direct causation involving the presence of less virulent strains of *H. pylori* and antibiotic resistance. Rather, the presence or absence of virulence factors may cause physiological effects which create an environment in which antibiotic resistant strains of *H. pylori* can flourish as outlined above[31]. As less virulent strains are less immunogenic, an inadequate delivery of antibiotics may reach infected areas in the stomach and as a result, antimicrobial resistant strains may be selected for in the population of less virulent strains. It has been shown that a *cagA-* strain may tend to acquire drug resistance *in vitro*[12]. Indeed, studies have shown that virulence factor genotype may also influence treatment outcome. A number of studies have reported the presence or absence of *cagA* and *vacA* as predictors of eradication of *H. pylori*[36,38-41]. Wang *et al*[40]conducted a meta-analysis of 25 studies and found that infection with *cagA* positive*,* *vacA* S1strains were associated with *H. pylori* eradication.

In conclusion, this study found that the *cagA* negative and *vacA* S1/M2 genotypes were the most dominant in *H. pylori* strains in Ireland. A surprisingly high rate of primary genotypic clarithromycin resistance was observed (50.5%), with a primary genotypic fluoroquinolone resistance rate of 15.2%. It was also found that there is a relationship between the less virulent strains of *H. pylori* (*cagA*-negative and *vacA* S2) and primary clarithromycin resistance. It is well known that the prevalence of antibiotic resistance is increasing worldwide while eradication rates of *H. pylori* are decreasing. The relationship between less virulent strains of *H. pylori* and presence of antibiotic resistance found herein could potentially be an avenue to explore in the effort to improve eradication rates.

**ARTICLE HIGHLIGHTS**

***Research background***

*H. pylori* causes chronic gastritis, gastric and duodenal ulcers, gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma. Disease outcome is related to both host and bacterial factors. Eradication is recommended in all symptomatic patients and those at risk of gastric cancer. However, eradication rates for current therapies are falling due to the emergence of antibiotic resistant *H. pylori* strains. *H. pylori* is a highly heterogeneous bacterium and its virulence varies geographically. Virulence factors contribute to the pathogenicity of the bacteria and have been suggested to influence treatment outcome.

***Research motivation***

In response to the increasing problem of *H. pylori* antibiotic resistance, local antibiotic resistance surveillance is recommended to guide clinicians in their choice of *H. pylori* therapy. Knowledge of local antimicrobial resistance rates and the prevalence of virulent infections will influence strategies for optimising the management of *H. pylori* infection.

***Research objectives***

This study aimed to provide an update on the prevalence of antibiotic resistance in Ireland, in particular for the antibiotics clarithromycin and fluoroquinolones. The virulence of the infecting strains was assessed by investigating *cagA* and *vacA* status. In addition the relationship between virulence factor status and antibiotic resistance was evaluated.

***Research methods***

DNA was extracted from antral and corpus biopsies obtained from *H. pylori*-infected patients. Genotyping for clarithromycin and fluoroquinolone-mediating mutations was performed using the Genotype HelicoDR assay. *CagA* and *vacA* genotypes were investigated using PCR and agarose gel electrophoresis.

***Research results***

Primary resistance to clarithromycin was high at 50.5%. Primary resistance to fluoroquinolones was 15.2%. Primary resistance to both antibiotics was 12.4%. A *cagA*-positive genotype was detected in 22.4% of patient samples. The dominant *vacA* genotype was S1/M2 at 44.8%, followed by S2/M2 at 26.7%, S1/M1 at 23.6% and S2/M1 at 4.8%. Primary clarithromycin resistance was significantly lower in *cagA*-positive strains than in *cagA*-negative strains (32% *vs* 56.3%). Similarly, in patients infected with more virulent *H. pylori* strains bearing the *vacA* s1 genotype, primary clarithromycin resistance was significantly lower than in those infected with less virulent strains bearing the *vacA* s2 genotype, (41% *vs* 77.8%). In summary, genotypic *H. pylori* clarithromycin resistance is high and *cagA*-negative strains are dominant in our population. Less virulent (*cagA*-negative and *vacA* S2-containing) strains of *H. pylori* are associated with primary clarithromycin resistance.

***Research perspectives***

Given the high rate of primary clarithromycin resistance detected in our study, the use of alternatives to clarithromycin-based triple therapy should be considered for first line *H. pylori* treatment in our cohort. In order to validate the association between less virulent strains and clarithromycin resistance, the influence of virulence factor genotype on treatment outcome should be assessed.

**ACKNOWLEDGMENTS**

## The authors would like to acknowledge the Health Research Board and also Mark Feighery, Ciara Treacy and Edwin Fahy for technical assistance.

**REFERENCES**

1 **Malfertheiner P**, Megraud F, O'Morain CA, Gisbert JP, Kuipers EJ, Axon AT, Bazzoli F, Gasbarrini A, Atherton J, Graham DY, Hunt R, Moayyedi P, Rokkas T, Rugge M, Selgrad M, Suerbaum S, Sugano K, El-Omar EM; European Helicobacter and Microbiota Study Group and Consensus panel. Management of Helicobacter pylori infection-the Maastricht V/Florence Consensus Report. *Gut* 2017; **66**: 6-30 [PMID: 27707777 DOI: 10.1136/gutjnl-2016-312288]

2 **Leja M**, Axon A, Brenner H. Epidemiology of Helicobacter pylori infection. *Helicobacter* 2016; **21** Suppl 1: 3-7 [PMID: 27531531 DOI: 10.1111/hel.12332]

3 **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]

4 **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]

5 **Megraud F**, Coenen S, Versporten A, Kist M, Lopez-Brea M, Hirschl AM, Andersen LP, Goossens H, Glupczynski Y; Study Group participants. 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]

6 **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]

7 **Fischbach L**, Evans EL. Meta-analysis: the effect of antibiotic resistance status on the efficacy of triple and quadruple first-line therapies for Helicobacter pylori. *Aliment Pharmacol Ther* 2007; **26**: 343-357 [PMID: 17635369 DOI: 10.1111/j.1365-2036.2007.03386.x]

8 **Broutet N**, Tchamgoué S, Pereira E, Lamouliatte H, Salamon R, Mégraud F. Risk factors for failure of Helicobacter pylori therapy--results of an individual data analysis of 2751 patients. *Aliment Pharmacol Ther* 2003; **17**: 99-109 [PMID: 12492738 DOI: 10.1046/j.1365-2036.2003.01396.x]

9 **Uotani T**, Miftahussurur M, Yamaoka Y. Effect of bacterial and host factors on Helicobacter pylori eradication therapy. *Expert Opin Ther Targets* 2015; **19**: 1637-1650 [PMID: 26245678 DOI: 10.1517/14728222.2015.1073261]

10 **Atherton JC**, Cao P, Peek RM Jr, Tummuru MK, Blaser MJ, Cover TL. Mosaicism in vacuolating cytotoxin alleles of Helicobacter pylori. Association of specific vacA types with cytotoxin production and peptic ulceration. *J Biol Chem* 1995; **270**: 17771-17777 [PMID: 7629077 DOI: 10.1074/jbc.270.30.17771]

11 **Rhead JL**, Letley DP, Mohammadi M, Hussein N, Mohagheghi MA, Eshagh Hosseini M, Atherton JC. A new Helicobacter pylori vacuolating cytotoxin determinant, the intermediate region, is associated with gastric cancer. *Gastroenterology* 2007; **133**: 926-936 [PMID: 17854597 DOI: 10.1053/j.gastro.2007.06.056]

12 **Taneike I**, Nami A, O'Connor A, Fitzgerald N, Murphy P, Qasim A, O'Connor H, O'Morain C. Analysis of drug resistance and virulence-factor genotype of Irish Helicobacter pylori strains: is there any relationship between resistance to metronidazole and cagA status? *Aliment Pharmacol Ther* 2009; **30**: 784-790 [PMID: 19604178 DOI: 10.1111/j.1365-2036.2009.04095.x]

13 **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**: 9214-9221 [PMID: 27895408 DOI: 10.3748/wjg.v22.i41.9214]

14 **Pastukh N**, Binyamin D, On A, Paritsky M, Peretz A. GenoType® HelicoDR test in comparison with histology and culture for Helicobacter pylori detection and identification of resistance mutations to clarithromycin and fluoroquinolones. *Helicobacter* 2017; **22**: [PMID: 29058343 DOI: 10.1111/hel.12447]

15 **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]

16 **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]

17 **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]

18 **Sanches BS**, Martins GM, Lima K, Cota B, Moretzsohn LD, Ribeiro LT, Breyer HP, Maguilnik I, Maia AB, Rezende-Filho J, Meira AC, Pinto H, Alves E, Mascarenhas R, Passos R, de Souza JD, Trindade OR, Coelho LG. Detection of Helicobacter pylori resistance to clarithromycin and fluoroquinolones in Brazil: A national survey. *World J Gastroenterol* 2016; **22**: 7587-7594 [PMID: 27672279 DOI: 10.3748/wjg.v22.i33.7587]

19 **Tanih 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]

20 **Macías-García F**, Llovo-Taboada J, Díaz-López M, Bastón-Rey I, Domínguez-Muñoz JE. High primary antibiotic resistance of Helicobacter Pylori strains isolated from dyspeptic patients: A prevalence cross-sectional study in Spain. *Helicobacter* 2017; **22**: [PMID: 28913872 DOI: 10.1111/hel.12440]

21 **Kuo YT**, Liou JM, El-Omar EM, Wu JY, Leow AHR, Goh KL, Das R, Lu H, Lin JT, Tu YK, Yamaoka Y, Wu MS; Asian Pacific Alliance on Helicobacter and Microbiota. Primary antibiotic resistance in Helicobacter pylori in the Asia-Pacific region: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 2017; **2**: 707-715 [PMID: 28781119 DOI: 10.1016/S2468-1253(17)30219-4]

22 **McNulty CA**, Lasseter G, Shaw I, Nichols T, D'Arcy S, Lawson AJ, Glocker E. Is Helicobacter pylori antibiotic resistance surveillance needed and how can it be delivered? *Aliment Pharmacol Ther* 2012; **35**: 1221-1230 [PMID: 22469191 DOI: 10.1111/j.1365-2036.2012.05083.x]

23 **O'connor A**, Taneike I, Nami A, Fitzgerald N, Murphy P, Ryan B, O'connor H, Qasim A, Breslin N, O'moráin C. Helicobacter pylori resistance to metronidazole and clarithromycin in Ireland. *Eur J Gastroenterol Hepatol* 2010; **22**: 1123-1127 [PMID: 20354442 DOI: 10.1097/MEG.0b013e328338e43d]

24 **Smith S**, Boyle B, Brennan D, Buckley M, Crotty P, Doyle M, Farrell R, Hussey M, Kevans D, Malfertheiner P, Megraud F, Nugent S, O'Connor A, O'Morain C, Weston S, McNamara D. The Irish Helicobacter pylori Working Group consensus for the diagnosis and treatment of H. pylori infection in adult patients in Ireland. *Eur J Gastroenterol Hepatol* 2017; **29**: 552-559 [PMID: 28350745 DOI: 10.1097/MEG.0000000000000822]

25 **O'Connor A**, Taneike I, Nami A, Fitzgerald N, Ryan B, Breslin N, O'Connor H, McNamara D, Murphy P, O'Morain C. Helicobacter pylori resistance rates for levofloxacin, tetracycline and rifabutin among Irish isolates at a reference centre. *Ir J Med Sci* 2013; **182**: 693-695 [PMID: 23625165 DOI: 10.1007/s11845-013-0957-3]

26 **Feliciano O**, Gutierrez O, Valdés L, Fragoso T, Calderin AM, Valdes AE, Llanes R. Prevalence of Helicobacter pylori vacA, cagA, and iceA Genotypes in Cuban Patients with Upper Gastrointestinal Diseases. *Biomed Res Int* 2015; **2015**: 753710 [PMID: 25945344 DOI: 10.1155/2015/753710]

27 **Pajavand H**, Alvandi A, Mohajeri P, Bakhtyari S, Bashiri H, Kalali B, Gerhard M, Najafi F, Abiri R. High Frequency of vacA s1m2 Genotypes Among Helicobacter pylori Isolates From Patients With Gastroduodenal Disorders in Kermanshah, Iran. *Jundishapur J Microbiol* 2015; **8**: e25425 [PMID: 26862378 DOI: 10.5812/jjm.25425]

28 **van Doorn LJ**, Schneeberger PM, Nouhan N, Plaisier AP, Quint WG, de Boer WA. Importance of Helicobacter pylori cagA and vacA status for the efficacy of antibiotic treatment. *Gut* 2000; **46**: 321-326 [PMID: 10673291 DOI: 10.1136/gut.46.3.321]

29 **Godoy AP**, Ribeiro ML, Benvengo YH, Vitiello L, Miranda Mde C, Mendonça S, Pedrazzoli J Jr. Analysis of antimicrobial susceptibility and virulence factors in Helicobacter pylori clinical isolates. *BMC Gastroenterol* 2003; **3**: 20 [PMID: 12911839 DOI: 10.1186/1471-230X-3-20]

30 **Khan A**, Farooqui A, Manzoor H, Akhtar SS, Quraishy MS, Kazmi SU. Antibiotic resistance and cagA gene correlation: a looming crisis of Helicobacter pylori. *World J Gastroenterol* 2012; **18**: 2245-2252 [PMID: 22611319 DOI: 10.3748/wjg.v18.i18.2245]

31 **Sugimoto M**, Yamaoka Y. Virulence factor genotypes of Helicobacter pylori affect cure rates of eradication therapy. *Arch Immunol Ther Exp (Warsz)* 2009; **57**: 45-56 [PMID: 19219527 DOI: 10.1007/s00005-009-0007-z]

32 **Boyanova L**, Markovska R, Yordanov D, Gergova G, Mitov I. Clarithromycin Resistance Mutations in Helicobacter pylori in Association with Virulence Factors and Antibiotic Susceptibility of the Strains. *Microb Drug Resist* 2016; **22**: 227-232 [PMID: 26618567 DOI: 10.1089/mdr.2015.0199]

33 **Elviss NC**, Owen RJ, Xerry J, Walker AM, Davies K. Helicobacter pylori antibiotic resistance patterns and genotypes in adult dyspeptic patients from a regional population in North Wales. *J Antimicrob Chemother* 2004; **54**: 435-440 [PMID: 15243025 DOI: 10.1093/jac/dkh343]

34 **Karabiber H**, Selimoglu MA, Otlu B, Yildirim O, Ozer A. Virulence factors and antibiotic resistance in children with Helicobacter pylori gastritis. *J Pediatr Gastroenterol Nutr* 2014; **58**: 608-612 [PMID: 24792628 DOI: 10.1097/MPG.0000000000000273]

35 **López-Brea M**, Martínez MJ, Domingo D, Sánchez I, Alarcón T. Metronidazole resistance and virulence factors in Helicobacter pylori as markers for treatment failure in a paediatric population. *FEMS Immunol Med Microbiol* 1999; **24**: 183-188 [PMID: 10378418 DOI: 10.1111/j.1574-695X.1999.tb01280.x]

36 **Liou JM**, Chang CY, Chen MJ, Chen CC, Fang YJ, Lee JY, Wu JY, Luo JC, Liou TC, Chang WH, Tseng CH, Wu CY, Yang TH, Chang CC, Wang HP, Sheu BS, Lin JT, Bair MJ, Wu MS; Taiwan Gastrointestinal Disease and Helicobacter Consortium. The Primary Resistance of Helicobacter pylori in Taiwan after the National Policy to Restrict Antibiotic Consumption and Its Relation to Virulence Factors-A Nationwide Study. *PLoS One* 2015; **10**: e0124199 [PMID: 25942450 DOI: 10.1371/journal.pone.0124199]

37 **van Doorn LJ**, Glupczynski Y, Kusters JG, Mégraud F, Midolo P, Maggi-Solcà N, Queiroz DM, Nouhan N, Stet E, Quint WG. Accurate prediction of macrolide resistance in Helicobacter pylori by a PCR line probe assay for detection of mutations in the 23S rRNA gene: multicenter validation study. *Antimicrob Agents Chemother* 2001; **45**: 1500-1504 [PMID: 11302817 DOI: 10.1128/AAC.45.5.1500-1504.2001]

38 **Suzuki T**, Matsuo K, Sawaki A, Ito H, Hirose K, Wakai K, Sato S, Nakamura T, Yamao K, Ueda R, Tajima K. Systematic review and meta-analysis: importance of CagA status for successful eradication of Helicobacter pylori infection. *Aliment Pharmacol Ther* 2006; **24**: 273-280 [PMID: 16842453 DOI: 10.1111/j.1365-2036.2006.02994.x]

39 **Wang D**, Li Q, Gong Y, Yuan Y. The association between vacA or cagA status and eradication outcome of Helicobacter pylori infection: A meta-analysis. *PLoS One* 2017; **12**: e0177455 [PMID: 28493953 DOI: 10.1371/journal.pone.0177455]

40 **Figura N**, Moretti E, Vaglio L, Langone F, Vernillo R, Vindigni C, Giordano N. Factors modulating the outcome of treatment for the eradication of Helicobacter pylori infection. *New Microbiol* 2012; **35**: 335-340 [PMID: 22842603]

**P-Reviewer:** Chiba T, Rodrigo L, Sugimoto M **S-Editor:** Wang XJ

**L-Editor:** **E-Editor:**

**Specialty type:** Gastroenterology and hepatology

**Country of origin:** Ireland

**Peer-review report classification**

Grade A (Excellent): 0

Grade B (Very good): B, B

Grade C (Good): C

Grade D (Fair): 0

Grade E (Poor): 0



**Figure 1** **Prevalence of clarithromycin-resistance according to *cagA* genotype.** A: All patients; B: Treatment naïve patients; C: Previously treated patients.

****

**Figure 2** **Prevalence of clarithromycin resistance according to *vacA* genotype.** A: All patients; B: Treatment naïve patients; C: Previously treated patients. Most virulent: S1/M1, S1/M2; Least virulent: S2/M1; S2/M2.



**Figure 3 Prevalence of fluoroquinolone-resistance according to *cagA* genotype.** A: All patients; B: Treatment naïve patients; C: Previously treated patients.



**Figure 4 Prevalence of fluoroquinolone-resistance according to *vacA* genotype.** A: All patients; B: Treatment naïve patients; C: Previously treated patients. Most virulent: S1/M1, S1/M2; Least virulent: S2/M1; S2/M2.

**Table 1 Polymerase chain reaction primers used in this study**

|  |  |  |  |
| --- | --- | --- | --- |
| Primer | Primer sequence | Gene | Product size (bp) |
| CAGA-F | 5’-GATAACAGGCAAGCTTTTGATG-3’ | *cagA* | 349 |
| CAGA-R | 5’-CTGCAAAAGATTGTTTGGCAGA-3’ |
| VA1-F | 5’- ATGGAAATACAACAACAAACACAC-3’ | *vacA* signal region | 259/286 (s1/s2) |
| VA1-R | 5’ – CTGCTTGAATGCGCCAAAC-3’ |
| VAG-F | 5’ – CAATCTGTCCAATCAAGCGAG-3’ | *vacA* middle region | 567/642 (m1/m2) |
| VAG-R | 5’- GCTTCAAAATAATTCCAAGG-3’ |

**Table 2** **Demographic and clinical characteristics of *H. pylori*-infected patients included in the study**

|  |  |
| --- | --- |
|  | **Number of gastric biopsy specimens *n* (%)** |
|  | **All patients****165 (100)** | **Treatment Naïve****105 (63.6)** | **Previously treated****60 (36.4)** |
| Gender |  |  |  |
| Female | 69 (41.8) | 31 (29.5) | 38 (63.3) |
| Male | 96 (58.2) | 74 (70.5) | 22 (36.7) |
| Age |  |  |  |
| Mean ± SD | 49.2 ± 15.8 | 50.3 ± 16.3 | 47.4 ± 14.7 |
| Histology findings |  |  |  |
| Chronic gastritis | 130 (78.8) | 78 (74.3) | 52 (86.7) |
| Intestinal metaplasia | 23 (13.9) | 16 (15.2) | 7 (11.7) |
| No data available | 11 (6.7) | 10 (9.5) | 1 (1.7) |
| Normal mucosa | 1 (0.6) | 1 (1.0) | 0 (0.0) |
| Endoscopic findings |  |  |  |
| Gastritis | 92 (55.8) | 57 (54.3) | 35 (58.3) |
| Normal | 32 (19.4) | 19 (18.1) | 13 (21.7) |
| Gastric/ Duodenal Ulcer  | 21 (12.7) | 15 (14.3) | 6 (10.0) |
| No data available | 17 (10.3) | 11 (10.5) | 6 (10.0) |
| Atrophic mucosa | 1 (0.6) | 1 (1.0) | 0 (0.0) |
| Other1 | 2 (1.2) | 2 (1.9) | 0 (0.0) |

1Other endoscopic findings: 1 intestinal metaplasia and erosion: 1 portal hypertensive gastropathy.

**Table 3** C**larithromycin resistance rates and the distribution of resistance-mediating mutations**

|  |  |  |
| --- | --- | --- |
| **Genotype** | **Number of gastric biopsy specimens** ***n* (%)** | ***P* value1** |
| **All patients****165 (100)** | **Treatment Naïve****105 (63.6)** | **Previously treated****60 (36.4)** |
| ClarithromycinS (WT) | 65 (39.4) | 52 (49.5) | 13 (21.7) | < 0.001 |
| ClarithromycinR | 100 (60.6) | 53 (50.5) | 47 (78.3) |
| Point mutations  |  |  |  |  |
|  | A2147G | 78 (78) | 44 (83) | 34 (72.3) | NS |
|  | A2146G  | 8 (8) | 3 (5.7) | 5 (10.6) | NS |
|  | A2146C | 6 (6) | 3 (5.7) | 3 (6.4) | NS |
|  | A2146C + A2147G  | 5 (5) | 3 (5.7) | 2 (4.3) | NS |
|  | A2146G + A2147G  | 2 (2) | 0 (0) | 2 (4.3) | NS |
|  | A2146G + A2146C  | 1 (1) | 0 (0) | 1 (2.1) | NS |

1Treatment-naïve versus previously treated patients (Fisher’s exact test). ClarithromycinS: Sensitive to clarithromycin; ClarithromycinR: Resistant to clarithromycin.

**Table 4** F**luoroquinolone resistance rates and the distribution of resistance-mediating mutations**

|  |  |  |
| --- | --- | --- |
| **Genotype** | **Number of gastric biopsy specimens*****n* (%)** | ***P* value1** |
| **All patients****165 (100)** | **Treatment Naïve****105 (63.6)** | **Previously treated****60 (36.4)** |
| FluoroquinoloneS (WT) | 132 (80) | 89 (84.8) | 43 (71.7) | NS |
| FluoroquinoloneR | 33 (20) | 16 (15.2) | 17 (28.3) |
| Point mutations |  |  |  |  |
|  | *gyr*91 D91Y | 18 (54.5) | 10 (62.5) | 8 (47.1) | NS |
|  | *gyr*91 D91N | 6 (18.2) | 2 (12.5) | 4 (23.5) | NS |
|  | *gyr*91 D91G | 2 (6.1) | 0 (0) | 2 (11.8) | NS |
|  | *gyr91* D91N + *gyr91* D91G | 2 (6.1) | 1 (6.3) | 1 (5.9) | NS |
|  | *gyr91* D91N +*gyr91* D91Y | 2 (6.1) | 1 (6.3) | 1 (5.9) | NS |
|  | *gyr87* N87K | 1 (3) | 1 (6.3) | 0 (0) | NS |
|  | *gyr87* N87K+ *gyr91* D91N + *gyr91* D91G | 1 (3) | 0 (0) | 1 (5.9) | NS |
|  | *gyr87* N87K+ *gyr91* D91N + *gyr91* D91G + *gyr91* D91Y | 1 (3) | 1 (6.3) | 0 (0) | NS |

1Treatment-naïve versus previously treated patients (Fisher’s exact test). FluoroquinoloneS: Sensitive to fluoroquinolones; FluoroquinoloneR: Resistant to fluoroquinolones.

**Table 5 Antimicrobial susceptibility results for both clarithromycin and fluoroquinolone**

|  |  |  |
| --- | --- | --- |
| **Genotype** | **Number of gastric biopsy specimens *n* (%)** | ***P* value1** |
| **All patients****165 (100)** | **Treatment Naïve****105 (63.6)** | **Previously treated****60 (36.4)** |
| Susceptible (to both)  | 60 (36.4) | 49 (46.6) | 11 (18.3) | < 0.05 |
| Resistant (to at least one) | 105 (63.6) | 56 (53.3) | 49 (81.6) |
| Susceptible/resistant to one  | 137 (83.0) | 92 (87.6) | 45 (75) | 0.05 |
| Resistant to both | 28 (17.0) | 13 (12.4) | 15 (25) |

1Treatment-naïve versus previously treated patients (Fisher’s exact test).

**Table 6** **Distribution of *H. pylori* virulence-factor genotypes among infected patients in Ireland fluoroquinolone**

|  |  |  |
| --- | --- | --- |
| **Genotype** | **(*n*) %** | ***P* value1** |
| **Overall****(*n* = 165)** | **Treatment naïve (*n* = 105)** | **Previous treatment (*n* = 60)** |
| *cagA* statusPositiveNegative | 37 (22.4)128 (77.6) | 25 (23.8)80 (76.2) | 12 (20)48 (80) | NS |
| *vacA* alleleS1S2 | 113 (68.5)52 (31.5) | 78 (74.3)27 (25.7) | 35 (58.3)25 (41.7) | < 0.05 |
| M1M2 | 47 (28.5)118 (71.5) | 31 (29.5)74 (70.5) | 16 (26.7)44 (73.3) | NS |
| S1/M1 | 39 (23.6) | 26 (24.8) | 13 (21.7) | NS |
| S1/M2 | 74 (44.8) | 52 (49.5) | 22 (36.7) | NS |
| S2/M1 | 8 (4.8) | 5 (4.8) | 3 (5.0) | NS |
| S2/M2 | 44 (26.7) | 22 (21.0) | 22 (36.7) | < 0.05 |

1Treatment-naïve versus previously treated patients (Fisher’s exact test).