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Contents

Contents

Contents

ABOUT COVER

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AIMS AND SCOPE

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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.

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ORIGINAL ARTICLE

Basic Study

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

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Abstract

AIM

To evaluate the association between virulence factor status and antibiotic resistance in *Helicobacter pylori* (*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; Fluoroquinolone; Clarithromycin; Virulence factor; VacA; CagA

Core tip: The management of *Helicobacter pylori* (*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.

INTRODUCTION
Helicobacter pylori (*H. 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 eradicaton 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 factors 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

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 1 Polymerase chain reaction primers used in this study

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 wk); 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 ℃ 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 ℃ 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 χ^2-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 for 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 *vs* 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*

Table 2 Demographic and clinical characteristics of *Helicobacter 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)
Other[1]	2 (1.2)	2 (1.9)	0 (0.0)

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

Table 3 Clarithromycin resistance rates and the distribution of resistance-mediating mutations

Genotype	Number of gastric biopsy specimens *n* (%)			*P* value[1]
	All patients 165 (100)	Treatment Naïve 105 (63.6)	Previously treated 60 (36.4)	
Clarithromycin[S] (WT)	65 (39.4)	52 (49.5)	13 (21.7)	< 0.001
Clarithromycin[R]	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

[1]Treatment-naïve versus previously treated patients (Fisher's exact test). Clarithromycin[S]: Sensitive to clarithromycin; Clarithromycin[R]: Resistant to clarithromycin.

= 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); χ^2 = 8.04; *P* = 0.004; Pearson χ^2 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); χ^2 = 4.5; *P* = 0.03; Pearson χ^2 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); χ^2 = 10.6; *P* = 0.001; Pearson χ^2 test; Figure 2A] and in those that were treatment-naïve [41% (*n* = 32/78) *vs* 77.8% (*n* = 21/27); χ^2 = 10.8; *P* = 0.0001; Pearson χ^2 test; Figure 2B], but not in patients that were previously treated (Figure 2C).

Table 4 Fluoroquinolone resistance rates and the distribution of resistance-mediating mutations

Genotype	Number of gastric biopsy specimens *n* (%)			*P* value[1]
	All patients 165 (100)	Treatment Naïve 105 (63.6)	Previously treated 60 (36.4)	
Fluoroquinolone[S] (WT)	132 (80.0)	89 (84.8)	43 (71.7)	
Fluoroquinolone[R]	33 (20.0)	16 (15.2)	17 (28.3)	NS
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 (0.0)	2 (11.8)	NS
*gyr*91 D91N + *gyr*91 D91G	2 (6.1)	1 (6.3)	1 (5.9)	NS
*gyr*91 D91N +*gyr*91 D91Y	2 (6.1)	1 (6.3)	1 (5.9)	NS
*gyr*87 N87K	1 (3.0)	1 (6.3)	0 (0.0)	NS
*gyr*87 N87K + *gyr*91 D91N + *gyr*91 D91G	1 (3.0)	0 (0.0)	1 (5.9)	NS
*gyr*87 N87K + *gyr*91 D91N + *gyr*91 D91G + *gyr*91 D91Y	1 (3.0)	1 (6.3)	0 (0.0)	NS

[1]Treatment-naïve versus previously treated patients (Fisher's exact test). Fluoroquinolone[S]: Sensitive to fluoroquinolones; Fluoroquinolone[R]: Resistant to fluoroquinolones.
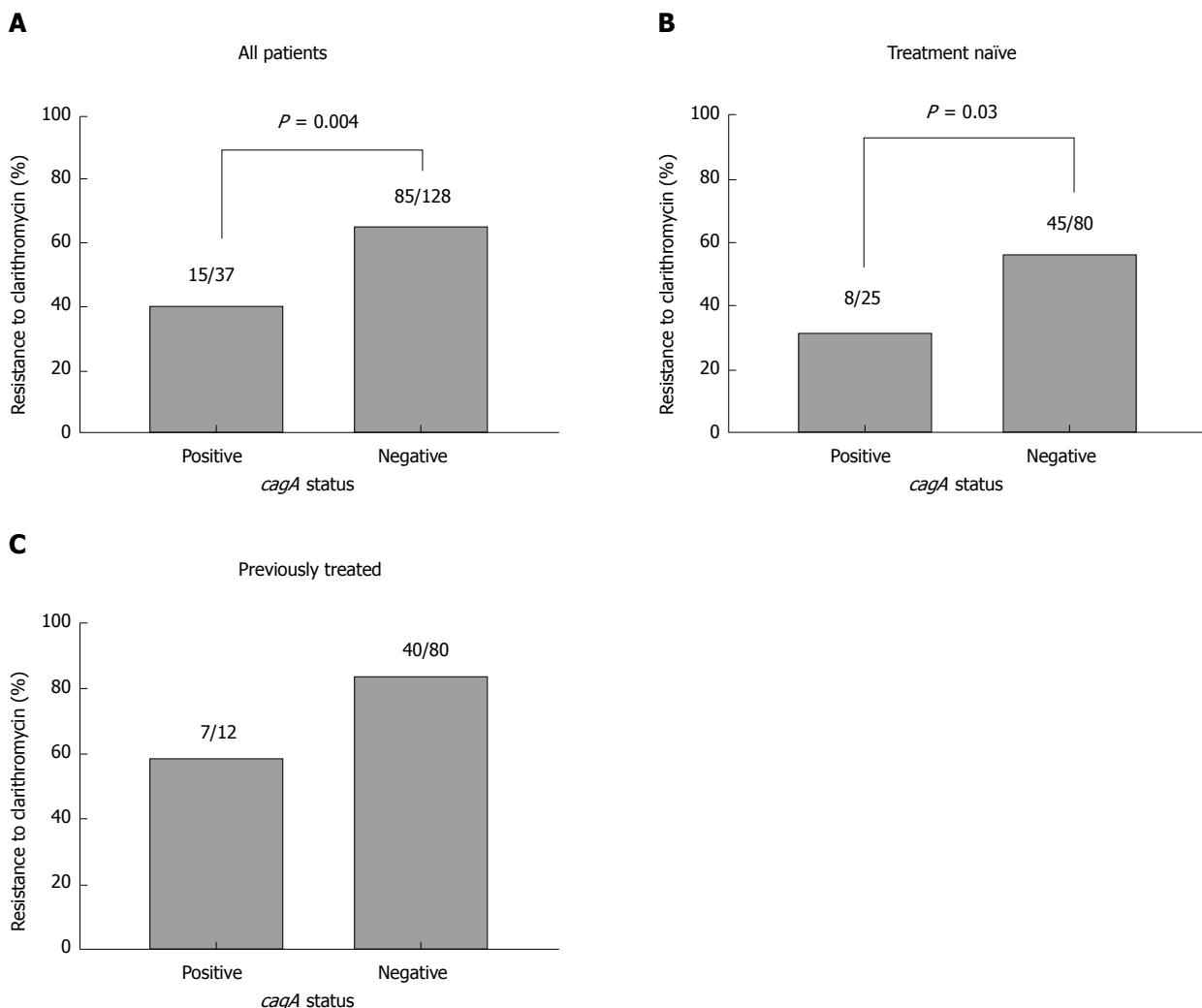
Figure 1 Prevalence of clarithromycin-resistance according to *cagA* genotype. A: All patients; B: Treatment naïve patients; C: Previously treated patients.

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 subdivided 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); χ^2 = 5.5; *P* = 0.02; Pearson χ^2 test; Figure 4A], this did not reach statistical

Table 5 Antimicrobial susceptibility results for both clarithromycin and fluoroquinolone

Genotype	Number of gastric biopsy specimens n (%)			P value[1]
	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)	
Resistant to both	28 (17.0)	13 (12.4)	15 (25.0)	0.05

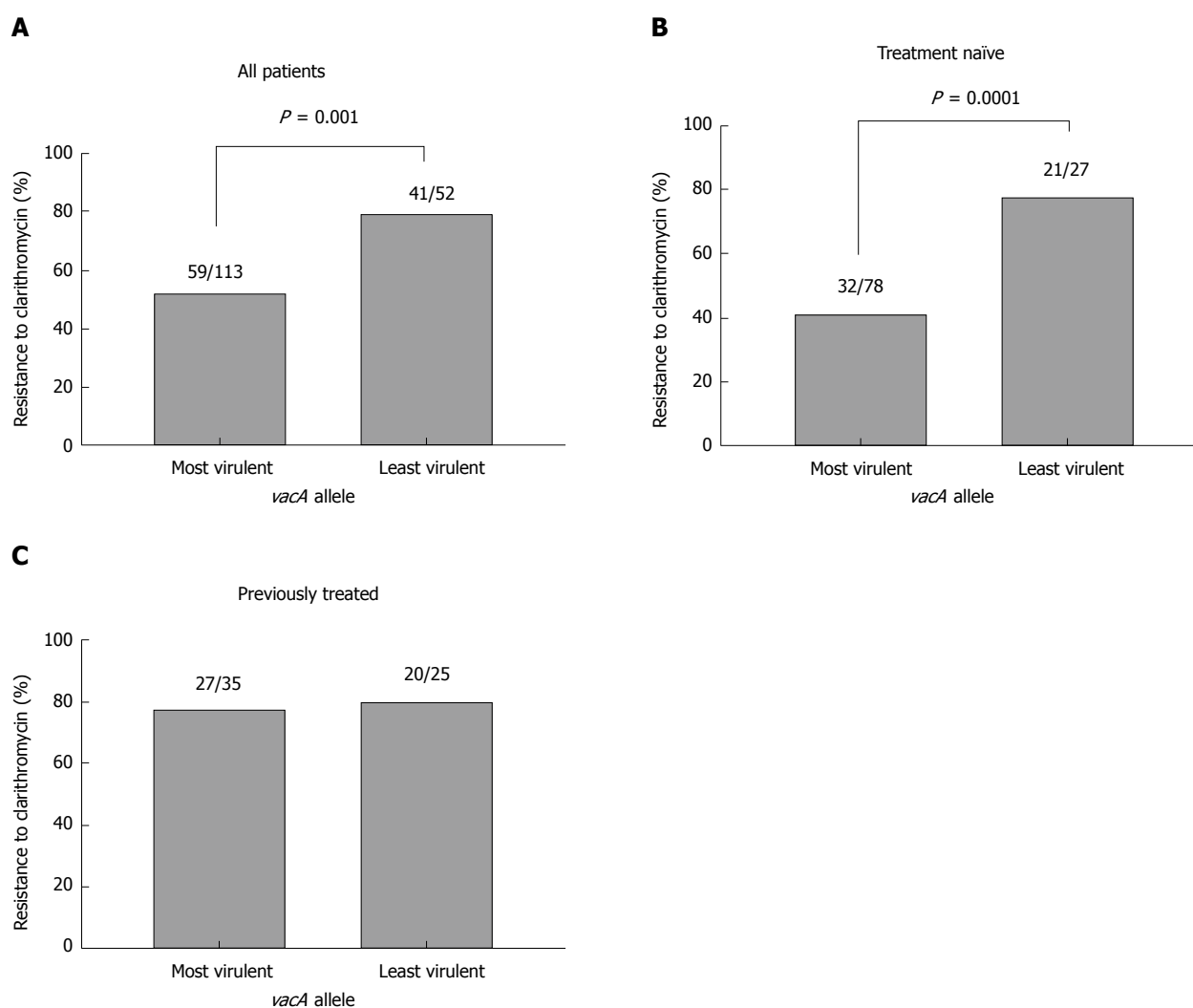[1]Treatment-naïve versus previously treated patients (Fisher's exact test).

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.

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

Table 6 Distribution of *Helicobacter pylori* virulence-factor genotypes among infected patients in Ireland *n* (%)				
Genotype	Overall (*n* = 165)	Treatment naïve (*n* = 105)	Previous treatment (*n* = 60)	*P* value[1]
cagA status				
Positive	37 (22.4)	25 (23.8)	12 (20)	NS
Negative	128 (77.6)	80 (76.2)	48 (80)	
vacA allele				
S1	113 (68.5)	78 (74.3)	35 (58.3)	
S2	52 (31.5)	27 (25.7)	25 (41.7)	< 0.05
M1	47 (28.5)	31 (29.5)	16 (26.7)	
M2	118 (71.5)	74 (70.5)	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

[1]Treatment-naïve versus previously treated patients (Fisher's exact test).
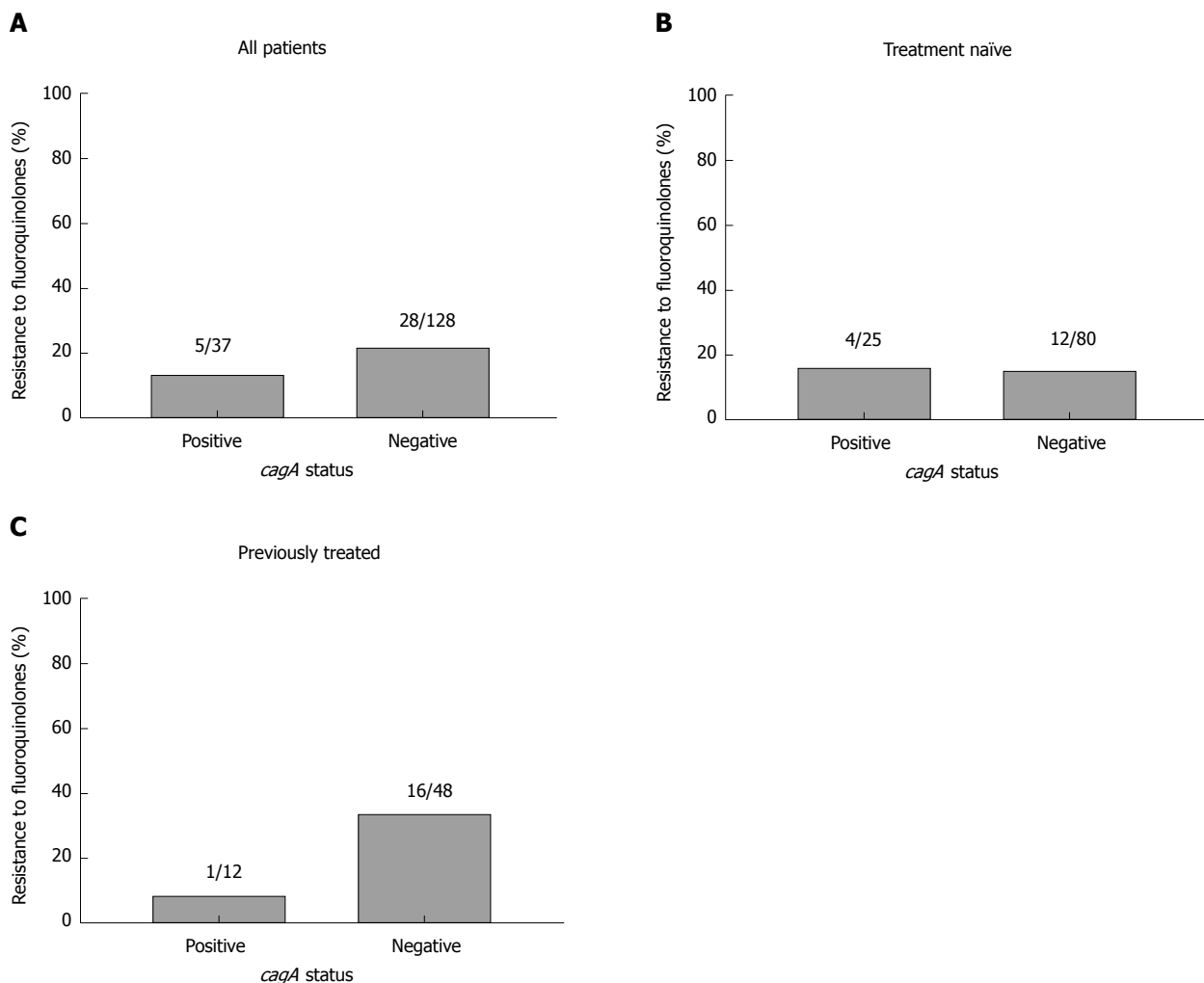
Figure 3 Prevalence of fluoroquinolone-resistance according to *cagA* genotype. A: All patients; B: Treatment naïve patients; C: Previously treated patients.

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

A

All patients

B

Treatment naïve

C

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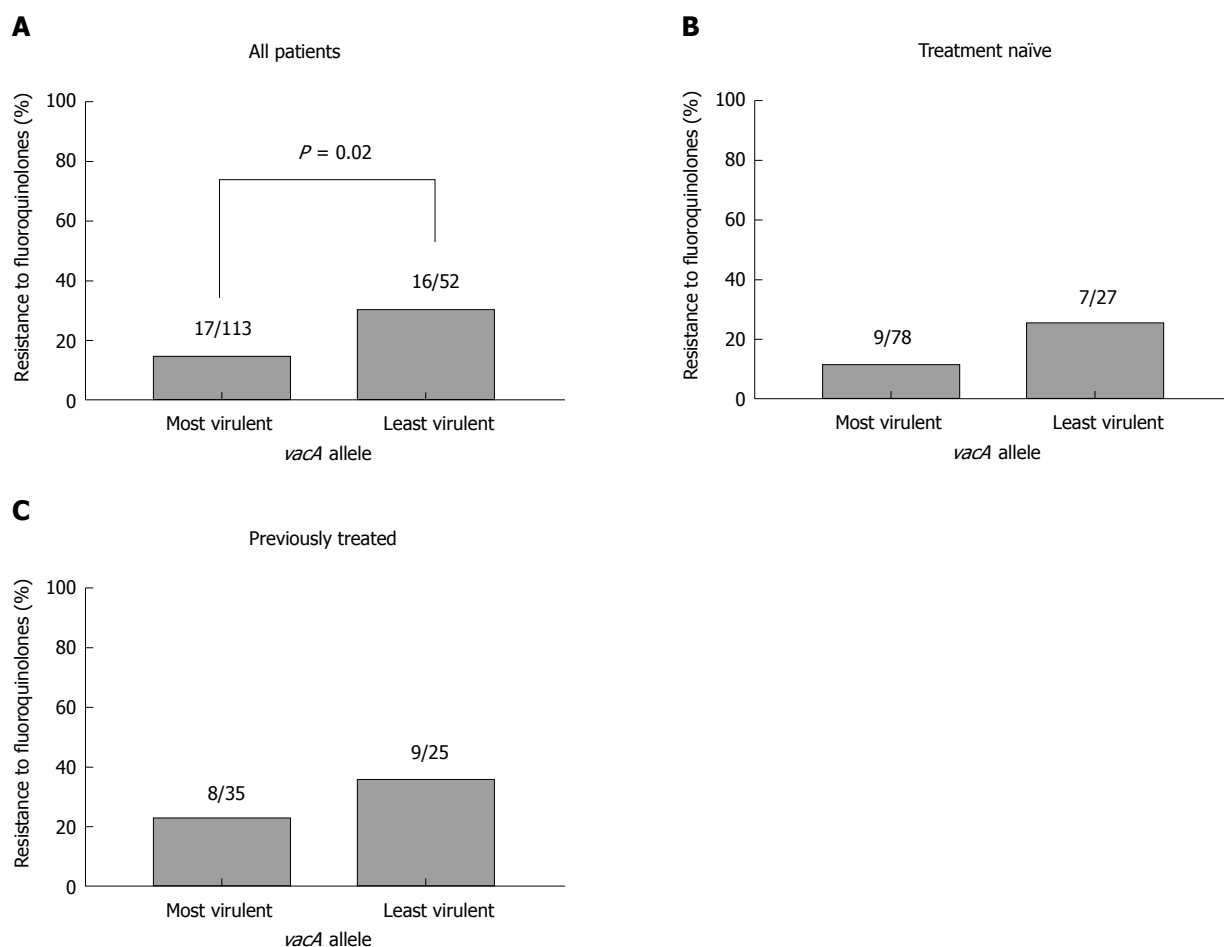
Figure 4 Prevalence of fluoroquinolone-resistance according to *vacA* genotype. A: All patients; B: Treatment naïve patients; C: Previously treated patients. Most virulent: S1/M1, S1/M2; Least virulent: S2/M1; S2/M2.

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, whose mechanisms of action 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*[36,38-40]. Wang *et al*[39] conducted a meta-analysis of 25 studies and found that infection with *cagA* positive*, vacA* S1 strains 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

Helicobacter pylori (*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.

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