Identification of antibiotic resistance patterns in *Helicobacter pylori* strains isolated from gastric biopsies using real-time PCR and genotypic analysis

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Abstract

Background *Helicobacter pylori* (*H. pylori*) is associated with dyspepsia, mucus-associated lymphoid tissue lymphoma, gastritis, and peptic ulcer disease. Treatment in Malta consists of triple therapy, which consists of a proton pump inhibitor and 2 of the antibiotics amoxicillin, clarithromycin, metronidazole and fluoroquinolones. We aimed to determine the resistance rates for clarithromycin and fluoroquinolones in patients with *H. pylori*, and its incidence, in patients undergoing an esophagogastroduodenoscopy (EGD) using real-time polymerase chain reaction (RT-PCR).

Methods Patients undergoing an EGD were recruited. A rapid urease test (RUT) was performed, and 4 gastric biopsies were also taken (2 from antrum, 2 from corpus) and analyzed using RT-PCR. Positive samples were tested for antibiotic resistance using amplification and reverse hybridization techniques.

Results Two hundred patients (mean age 53.6 [range 20-92] years; 53.1% female) were recruited; the majority were (78%) non-smokers. *H. pylori* was identified in 21.0% of the patients. Fluoroquinolone resistance was detected in 21.4% of the patients. Clarithromycin resistance was observed in 26.2%, with dual resistance identified in 4.8% of the patients. A high concordance was present with patients testing negative for *H. pylori* with both RUT and RT-PCR (94.3%). Only 57.6% of patients tested positive with both tests. However, 92.9% of RT-PCR positive patients had a positive genotypic HelicoDR test.

Conclusions This data demonstrates a high rate of *H. pylori* resistance to both clarithromycin and fluoroquinolones. These should be avoided when treating *H. pylori* by utilizing different treatment regimes. Furthermore, we derived important data on the role of RT-PCR, which may be implemented in routine clinical practice.

Keywords *Helicobacter pylori*, antibiotic resistance, clarithromycin, fluoroquinolones, genotype HelicoDR

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Introduction

Helicobacter species are Gram-negative helical-shaped micro-organisms. *Helicobacter pylori* (*H. pylori*) has the highest

clinical importance [1], as it leads to intestinal metaplasia and dysplasia [2]. It is classified as a Class 1 carcinogen [3].

Empirical treatment according to hospital guidelines consisted of clarithromycin triple therapy for 10-14 days. This consists of a proton pump inhibitor (PPI), clarithromycin, and amoxicillin or metronidazole. It is recommended in regions where clarithromycin resistance is known to be <15% for *H. pylori* and for patients with no recent macrolide exposure. In areas where resistance is higher, or patients have been treated with macrolides, bismuth-based quadruple therapy (BQT) is recommended. BQT consists of a PPI, bismuth, tetracycline, and a nitroimidazole for the same duration and can be considered as a first-line treatment.

In regions with high clarithromycin and metronidazole resistance rates and where bismuth would not be available, prescribed regimes include non-bismuth quadruple therapies (amoxicillin, tetracycline, rifabutin and furazolidone), sequential therapy (PPI and amoxicillin, followed by a PPI,

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clarithromycin and a nitroimidazole), and hybrid therapy (PPI and amoxicillin followed by a PPI, amoxicillin, clarithromycin and a nitroimidazole) [4].

Eradication failures are encountered primarily due to antibiotic resistance [5]. Although *H. pylori* exhibits low resistance to amoxicillin [6], resistance to clarithromycin and fluoroquinolones is increasing. This can be attributed to the overuse of antibiotics for other infections [7,8]. Resistance to metronidazole can be overcome by increasing the dosage, duration and frequency of treatment [4]. The long-lasting nature of *H. pylori* infection in the gastric mucosa makes it susceptible to antibiotics that can attain high concentrations in the gastric mucosa for an extended duration [9]. Thus, the World Health Organization has classified it as a priority Group 2 organism for drug development [10].

Locally, treatment failures have been observed. No studies about antibiotic resistance patterns in *H. pylori* had ever been conducted and there was a lack of knowledge about resistance. Current regional practice for the management of *H. pylori* adopts the “test and treat” strategy. Identification of *H. pylori* infection is performed through the rapid urease test (RUT) at endoscopy. Clarithromycin-based triple therapy is prescribed with positive results, whilst metronidazole and levofloxacin are second-line drugs. Any false-positive results lead to unnecessary costs and increased drug resistance.

The primary aims of this study were to: 1) determine primary resistance rates for clarithromycin and/or fluoroquinolones in *H. pylori* strains using reverse hybridization techniques; and 2) determine the incidence of *H. pylori* in Maltese patients undergoing an esophagogastroduodenoscopy (EGD) using real-time polymerase chain reaction (RT-PCR). The secondary objective for this study was to analyze any differences in detection when using the RUT test at endoscopy versus RT-PCR for *H. pylori* DNA in gastric biopsies.

Patients and methods

Patient selection, data collected and ethics

Patients attending the Endoscopy Unit were prospectively recruited and patient consent was obtained prior to the study. The main inclusion criterion was age 16 years or above. All patients were of Caucasian race. Exclusion criteria were: previous testing and/or treatment for *H. pylori* and use of antibiotics in the preceding 3 months. The presence of *H. pylori* DNA detected during testing was another inclusion criterion. Four gastric biopsies were collected per patient, 2 from the antrum and 2 from the corpus. A further 2 samples were collected for the RUT, one from the antrum and one from the body. Samples from the antrum were taken 5 cm proximal to the pylorus [11]. Biopsies were placed in 3 mL of 0.9% sterile saline in a sterile glass tube. All biopsies were kept at 4°C until processed. Data collected included age, sex, smoking status, excess intake of alcohol, intake of PPIs, and the result of the RUT. Indications for endoscopy are included in Table 1. The study was performed from February till September 2018.

Table 1 Indications for esophagogastroduodenoscopy

Indication	n = %
Dyspepsia	47
GERD	28
Iron deficiency anemia	15.5
Weight loss	5
Dysphagia	1
Work up for celiac disease	3.5
Total	100

GERD, gastroesophageal reflux disease

Ethical approval had been obtained from the University of Malta Research Ethics committee in November 2017.

Tissue digestion

All 4 biopsies were combined in a 1.5 mL micro-centrifuge tube to which 180 µL of tissue lysing buffer – Buffer ATL – supplied (QIAGEN®) were added. Samples were vortexed for 10 sec with subsequent addition of 20 µL of Proteinase K and vortex. All processed biopsies were stored at 56°C for a minimum of 48 h. Occasional vortex was also required during incubation to encourage lysis. All digested samples were stored at 4°C until further processing [12].

DNA extraction

Automated DNA extraction using the Qiagen QIAcube was done using the standardized tissue protocol of the QIAamp DNA mini kit (Qiagen Germany) [12]. All samples were re-heated to 56°C to allow Buffer ATL precipitates to dissolve. Samples were vortexed for 15 sec and 200 µL of the digested sample was pipetted into a second 1.5 mL micro-centrifuge tube, to which 10 µL of an *H. pylori* internal control provided with the RT-PCR kit was added. Sample lysis was carried out using Proteinase K and Buffer AL followed by DNA binding and washing. Elution of 100 µL of DNA was performed in Buffer AE. All eluted DNA was stored at -20°C [12].

RT-PCR using the Amplisens® *H. pylori*-FRT PCR kit

Amplification of genome specific regions in *H. pylori* using appropriate primers allowed identification of *H. pylori*. A master mix was prepared consisting of 10 µL of PCR Mix-1-FL, 5 µL of PCR Mix-2-FRT and 0.5µL of hot-start TaqF polymerase. Hot-StarTaqF polymerase was used to reduce the occurrence of non-specific reactions. Pipetting errors were compensated. DNA used per sample was 10 µL, leading to a total reaction volume of 25 µL per sample.

A positive and negative control were used with every RT-PCR run to ensure consistent results. All samples and controls

were pipetted into 0.1 mL disposable, capped polypropylene PCR strip tubes. These were loaded onto a Rotor-Disc and placed in the Rotor-Gene-Q (QIAGEN®). Fluorescent-labelled probes bound to the amplification product emitted a fluorescent signal, allowing *H. pylori* identification. Positive results were determined if a cycle threshold (*Ct*) value up to 38.00 was obtained. A positive control of amplification was used, while the negative template control consisted only of the reaction mixture [13]. A positive control strain was not utilized as positive and negative controls of amplification always gave consistent results.

Genotype HelicoDr – amplification and reverse hybridization technique for antibiotic resistance in *H. pylori*

All RT-PCR positive samples were tested for resistance to clarithromycin and/or fluoroquinolones using the genotype HelicoDr assay (n=42). Mutations – N87K in the *Gyr87* codon and mutations D91N, D91G, D91Y responsible for fluoroquinolone resistance in the *Gyr91* codon were identified for the *GyrA* gene. Mutations A2146G, A2146C and A2147G were identified for the 23srRNA (*Rrl*) gene responsible for clarithromycin resistance (GenBank Accession Number Nc_000915). DNA extracted was used. Multiplex PCR amplification using supplied biotinylated primers was performed. The amplicon was then used for reverse hybridization.

Reverse hybridization was done at 45°C using a vibrating incubator (TwinCubator, Hain Lifescience, Germany). Denaturation of the amplicon and hybridization of the denatured single-stranded biotin-labeled amplicon to membrane-bound DNA probes on coated strips were performed. Subsequent washing with stringent reagent, addition of a streptavidin/alkaline phosphatase conjugate, and addition of an alkaline phosphatase-mediated substrate allowed bands to become visible. The substrate concentrate was vortexed slightly to achieve a homogenous solution. Sensitivity or resistance of *H. pylori* to clarithromycin and/or fluoroquinolones was interpreted according to the bands obtained. The intensity of hybridization bands was compared to the amplification control band. Bands with a stronger intensity were interpreted. Only test strips that had both conjugate control and amplification control bands were interpreted, as per standard operating procedures [14].

Statistical analysis

Based on previous local studies [15,16], the sample size required for *H. pylori* detection within a cohort with a 95% degree of confidence was determined to be 200 patients. The identification of at least one resistant strain of *H. pylori* in gastric biopsies through genotypic mutations to either one or both antibiotics was enough to satisfy our primary objective. All data, except age, was categorical. Therefore, Pearson's chi-squared test was used. Pearson's chi square test was carried

out to identify any associations between variables (Table 2). RUT results were compared with RT-PCR results. Age was transformed into a categorical variable by the generation of age groups. A 0.05 level of significance was taken. P-values of <0.05 obtained showed that a statistically significant association was present. Fisher's exact test was also used. In addition, descriptive statistical techniques were used to observe associations graphically. Data were analyzed using SPSS software version 22 (IBM, New York, United States) using a Mac OS.

Quality control

All analyzers used had been previously calibrated according to standard laboratory protocols. All techniques used were validated. Controls were used to ensure valid results. The protocols were performed according to standard operating procedures.

Results

Two hundred patients were recruited (females: 53.1%), with 78% of patients being non-smokers. Excess alcohol intake was observed in 4.1% of male patients. Fig. 1 demonstrates the patients' characteristics in terms of age, smoking and alcohol consumption. The mean age was 53.6 years (SD ± 16.3 years), with the median age being 56 years.

***H. pylori* detection**

H. pylori was detected in 21.0% of patients tested by RT-PCR. In comparison, 29.5% of patients had a positive RUT at endoscopy. A positive RUT and a positive RT-PCR result were observed in 17.0% of patients, while 12.5% of patients had a positive RUT result but a negative RT-PCR result and 4.0% had a negative RUT but a positive RT-PCR. *H. pylori* detected by RT-PCR was again detected by the genotype HelicoDR kit in 92.9% of those patients. In 7.1% of RT-PCR positive patients, a negative *H. pylori* result was obtained with the genotype HelicoDR kit (Table 3). Incidentally, a negative RUT at endoscopy was also obtained.

Antibiotic resistance in *H. pylori*

Among a total of 42 positive patients, wild-type (WT) sensitive *H. pylori* strains were detected in 50.0% of patients. Total clarithromycin resistance was detected in 26.2% of positive patients and total fluoroquinolone resistance in 21.4% (Table 4). Heterogeneous strains were detected in 26.2% of positive patients, as more than one hybridization band was detected with both genes tested. Hetero-resistance was also observed. This signifies the strain being tested was expressing both WT and mutated bands, or that the patient was infected with more than one strain of *H. pylori*. Table 4

Table 2 Demographic features of *Helicobacter pylori* (*H. pylori*)-positive patients

Independent variable	Demographics	Total Subjects tested	<i>H. pylori</i>		P-value
			Positive (%)	Negative (%)	
Age					0.067
	40 years or less	48	6 (14.3)	42 (26.6)	
	41-55 years	50	7 (16.7)	43 (27.2)	
	56-65 years	46	14 (33.3)	32 (20.3)	
	66 or more years	56	15 (35.7)	41 (25.9)	
Sex					0.797
	Male	94	19 (20.2)	75 (79.8)	
	Female	106	23 (21.7)	83 (78.3)	
Smoking status					0.020
	Non-Smoker	156	27 (17.3)	129 (82.7)	
	Smoker	26	7 (26.9)	19 (73.1)	
	Ex-Smoker	18	8 (44.4)	10 (55.6)	
Excess alcohol intake					0.003
	Yes	8	5 (62.5)	3 (37.5)	
	No	192	37 (19.3)	155 (80.7)	
Rapid urease test					<0.001
	Positive	59	34 (57.6)	25 (42.4)	
	Negative	141	8 (5.7)	133 (94.3)	
Use of PPIs					0.453
	Yes	104	24 (23.1)	80 (76.9)	
	No	96	18 (18.8)	78 (81.3)	

PPIs, proton pump inhibitors

Table 3 Correlation of results between different methods of testing for *Helicobacter pylori* (*H. pylori*) identification

Type of test	Total number of patients (n=200)	Percentage (%)
RUT positive and <i>H. pylori</i> RT-PCR positive	34	17.0
RUT positive and <i>H. pylori</i> RT-PCR negative	25	12.5
RUT negative and <i>H. pylori</i> RT-PCR positive	8	4.0
RUT negative and <i>H. pylori</i> RT-PCR negative	133	66.5
	Total number of patients (n=42)	Percentage (%)
RT-PCR positive and genotype HelicoDR positive	39	92.9
RT-PCR positive and genotype HelicoDR negative	3	7.1

RUT, rapid urease test; RT-PCR, real-time polymerase chain reaction

shows the percentage of hetero-resistant strains detected for clarithromycin and fluoroquinolones.

Genotype detection

Sensitivity or resistance was determined using the genotype HelicoDR molecular assay. This analysis of the *Gyr87* and *Gyr91* codon in the *GyrA* gene identified fluoroquinolone resistance. The most common allele for *Gyr87*, identified in 20 patients, was WT-1, showing sensitivity to fluoroquinolones. Three mutation probes, mutation 1 - D91N, mutation 2 - D91G and mutation 3 - D91Y, were also identified for *Gyr91*.

Sensitivity or resistance to clarithromycin is identified through mutations in the 23srRNA gene. The WT band for the 23s rRNA gene indicating sensitivity to clarithromycin was identified in 34 patients. Mutation band 3 (mutation A2147G) for clarithromycin resistance was the most common allele. Fig. 2 and Table 4 show the genotypes detected in all of our patient cohort. Information related to genotypes detected is interpreted in comparison to age groups and sex.

H. pylori was found in patients who were either smokers (26.9%) or ex-smokers (44.4%), significantly more frequently than in non-smokers (17.3%) ($P=0.020$) (Table 2). From our total patient cohort who consumed excess alcohol ($n=8$), 62.5% tested positive for *H. pylori* ($n=5$). Statistically, this was significantly higher than *H. pylori*-positive patients who

Table 4 Tabulation of different genotypes detected showing antibiotic resistance patterns between sexes and age groups

Genotype detected		<i>H. pylori</i> +ve patients (n=42)	Percentage (%)	Male (n=42)	Female (n=42)	Mean age of patients in years
Sensitive <i>H. pylori</i> strains		21	50	10	11	57.3
Total clarithromycin resistance		11	26.2	3	8	63.5
A2146G	MUT1	3	7.1	1	2	54.3
A2146C	MUT2	2	4.8	0	2	71.5
A2147G	MUT3	7	16.7	2	5	66.6
A2146C+A2147G	MUT2+MUT3	1	2.4	0	1	73
Hetero-resistance to clarithromycin		6	14.3	2	4	61.7
Total fluoroquinolone resistance		9	21.4	4	5	58.4
N87K	<i>GyrA</i> 87 MUT	3	7.1	1	2	42.3
D91N	<i>GyrA</i> 91 MUT 1	3	7.1	0	3	63.7
D91G	MUT 2	1	2.4	1	0	81
D91K	MUT 3	2	4.8	2	0	65
Hetero-resistance to fluoroquinolones		4	9.5	2	2	52.5
Dual resistance to clarithromycin and fluoroquinolones		2	4.8	1	1	68.5
<i>H. pylori</i> negative by genotype HelicoDr		3	7.1	3	0	59.3

H. pylori, *Helicobacter pylori* **Figure 1** Patient demographics. Graph showing the characteristics of the patients with regards to age, smoking status and alcohol consumption

did not consume excess alcohol (n=37/200; 19.3%; $P=0.003$), (Table 2).

When comparing the results of the RUT with results from RT-PCR, a negative result was obtained in 94.3% of total patients tested. Positive RT-PCR results were also compared with the RUT results, and only 57.6% of patients had a positive result with both tests. The remainder either had a positive RT-PCR result and a negative RUT result, or a positive RUT with

a negative RT-PCR result. However, a statistically significant association was still found between both tests ($P<0.001$) (Table 2).

H. pylori was present in 21.7% of females and 20.2% of males ($P=0.797$). No sex-related difference was detected. The highest percentage of *H. pylori* positive results was obtained in patients over 66 years old (35.7%). Patients below the age of 40 years had the lowest percentage (14.3%). Fisher's test also

Figure 2 Prevalence of different mutations. The different mutations for clarithromycin and fluoroquinolone resistance as detected in *Helicobacter pylori*-positive patients

showed that patients above the age of 55 years were more likely to have *H. pylori* infection with antibiotic resistance ($P=0.0264$) (Table 4). No statistically significant association was observed between intake of PPIs and detection of *H. pylori* through RT-PCR ($P=0.453$).

Discussion

Our study demonstrated that the prevalence of *H. pylori* among patients who require an EGD was 21%. Among patients who tested positive for *H. pylori*, 26.2% exhibited resistance to clarithromycin while a further 21.4% exhibited resistance to fluoroquinolones. A small percentage, 4.8%, exhibited resistance to both antimicrobials.

The prevalence of *H. pylori* in our cohort correlates with local data from 2009, which had demonstrated a prevalence of 38% from histology and RUT [15]. A 2017 study using only RUT also demonstrated a 15% prevalence [16]. This study also demonstrated that prevalence increased with age, being highest in those above 66 years of age. This mirrors data from international studies [17,18,19] and the above supports the validity of our study. Although resistance was more common in people above 55 years, this was not statistically significant. Eighty percent (80%) of patients who were resistant to fluoroquinolones were in the 56–65 age group, while 66.7% of patients resistant to clarithromycin were above 66 [20]. Furthermore, no association between the use of PPIs and

antibiotic resistance was observed. However, it is thought that the use of high dosage PPIs could improve eradication rates, although this was found to be more effective in high-metabolizing PPI patients [4].

The RUT is an efficient way to determine the presence or absence of *H. pylori* infection [21]. In our study, 42.4% of the total positive RUT observed at endoscopy had a negative result with RT-PCR. Locally, all positive RUTs are treated empirically. Hence, these patients may be treated unnecessarily. A possible reason for this is not following the manufacturer's instructions and reading the RUT late [21]. However, in our study we made sure that the specific instructions were followed. The presence of other urease-producing organisms, such as members of *Enterobacteriales*, or *Staphylococcus aureus*, can produce a false-positive RUT [4]. Meanwhile the use of PPIs or the presence of blood can give false-negative results. Use of antibiotics might have led to false-negative RUT results; however, all patients were questioned thoroughly regarding recent antibiotic usage. Additionally, 5.7% of all negative RUT results had a positive RT-PCR result. This signifies that these patients were potentially infected and not treated. The use of PPIs prior to endoscopy can also lead to a negative RUT and histology result. This is not the case with molecular techniques [22,23].

Interestingly, 3 of the patients with a positive RT-PCR result but a negative RUT had a very high Ct value with RT-PCR (35.85, 34.46 and 37.07, respectively). Such high values signify a low bacterial load. This explains the negative RUT, as a minimum of 10,000 organisms is required [21]. Specimens tested by RT-PCR were taken from adjacent to those tested by RUT. These patients

also had a negative result with the genotype HelicoDR kit, suggesting that this assay might have a higher limit of detection, even though no international data are available. Considering the incidence of false-positive and false-negative results, the identification of *H. pylori* should be based on 2 or more tests [21,24]. However, the positive correlation between the RT-PCR and genotype HelicoDR suggests that the former test may be a more appropriate and reliable test to perform during endoscopy.

H. pylori was found in smokers and ex-smokers statistically more frequently than in non-smokers ($P=0.020$). International data regarding smoking are conflicting, with only some studies confirming this association [25–30]. Though one might note a significant association between excessive intake of alcohol and prevalence of *H. pylori* infection, one should consider that there were only 8 patients who consumed excess alcohol. This represents a significant bias. There are dissimilarities in the literature regarding the effects of excess alcohol [31,32].

Culture of *H. pylori* is not routine at our laboratory. Furthermore, it is laborious to grow and specific transport conditions are required [22,30]. The use of the genotype HelicoDR molecular assay allowed the identification of *H. pylori* and its genotypic expression of resistance. Genotype HelicoDR has a higher sensitivity (80.3%) and specificity (75.0%) when compared to culture, and comparable sensitivity (74.2%) and specificity (75.0%) when compared to histology [33]. Genotypic expression was not compared to phenotypic expression of resistance in this study; however, studies report a good correlation [34,35].

The advantage of culture lies in the identification of resistance to other antimicrobials, including metronidazole, amoxicillin and tetracyclines [34], whilst the identification of hetero-resistant strains is easier to detect via molecular techniques [22]. Our results have indicated the presence of hetero-resistance to both antimicrobials in several patients. Hetero-resistance is important, as it can show either the presence of 2 different populations of *H. pylori*, with different sensitivities, or the same strain expressing both the normal gene and the mutation. In the first scenario, *H. pylori* is partially eradicated.

Current guidance suggests avoiding the prescription of clarithromycin in areas where resistance levels are higher than 15% [4]. Our findings indicate high levels of primary resistance to both clarithromycin (26.2%) and fluoroquinolones (21.4%), suggesting that the use of standard empirical triple therapy should be altered. Our data correlate with a recent meta-analysis stating that resistance to clarithromycin and levofloxacin has increased worldwide [36,37]. Data from North America demonstrate resistance rates of approximately 30% for both antibiotics [6,37].

Studies suggest that therapy regimens should be tailored based on genotypic results. This has exhibited an eradication rate of more than 80%. Patients who exhibit only the WT strain for both the 23s rRNA (*Rrl*) and the *Gyr* alleles would be eligible for empirical triple therapy treatment [39,40]. Patients who exhibit mutations in the 23s rRNA (*Rrl*) allele but WT strains for the *Gyr* allele would be eligible for first-line fluoroquinolone-based therapies. Patients who exhibit dual resistance would be eligible for other non-clarithromycin

and non-fluoroquinolone therapies, or a salvage regimen containing amoxicillin and rifabutin or a PPI for 10-days [41]. In our study, 42 patients were positive for *H. pylori* DNA with RT-PCR. Eighteen patients showed resistance to either clarithromycin or fluoroquinolones or both, whilst a further 3 patients gave a negative *H. pylori* result. Thus, 42.8% of our tested population showed resistance, and, if treated with therapies containing either/or of these antibiotics, they would not exhibit an eradication rate of more than 80%. Therefore, it can be concluded that only 50% of our *H. pylori* positive patients can be treated safely with such therapies, assuming our patients are to have more than 80% chance of total recovery.

Based on the above, it would be wiser to eliminate clarithromycin-based therapies and utilize non-bismuth quadruple concomitant therapy or BQT. Only concomitant therapy can be prescribed in Malta, as bismuth compound is not available for treatment. Although clarithromycin is present, the presence of a nitroimidazole would still render the treatment effective. Though metronidazole resistance levels are not known in Malta, and were not within the scope of study in this analysis, metronidazole resistance can be overcome by increasing treatment dosage, dosing interval and duration [4].

Several limitations were encountered during this study. Recruitment was difficult, owing primarily to the small Maltese population and previous exposure to antibiotics in the preceding 3 winter months, leading to their exclusion. We also did not analyze all patients with all 3 methods (RUT, RT-PCR and genotype HelicoDR). Further validation of the RT-PCR in detection of *H. pylori* would need to be used in conjunction with RUT, genotype HelicoDR, as well as noninvasive testing such as fecal antigen testing. As culture and sensitivity is not routine, this was also a major limitation, as the phenotypic expression of resistance and the corresponding minimal inhibitory concentration of clarithromycin and fluoroquinolones could not be reported. Constraints of funding, time and lack of knowledge about the routine culture of *H. pylori* affected our study, as PCR results could not be confirmed via antibiotic susceptibility testing. Time constraints also precluded the use of the Sydney protocol during biopsy collection.

In conclusion, this study has demonstrated the unknown antibiotic resistance levels of *H. pylori* strains for clarithromycin and fluoroquinolones. Furthermore, testing for *H. pylori* with RT-PCR will allow the correct identification of patients with this infection. This avoids unnecessary prescriptions and identification of previously false-negative patients (RUT and histology) who would have been taking PPIs.

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Summary Box

What is already known:

- *Helicobacter pylori* (*H. pylori*) is statistically more significantly found in patients above 55 years of age. This was also observed in our study ($P=0.0264$)
- Smokers were more likely to have *H. pylori* than non-smokers ($P=0.020$), confirming international literature
- There is an increase in antibiotic resistance to clarithromycin and fluoroquinolones world wide
- Triple therapy should be avoided in areas of high antibiotic resistance as per guidelines stated in the Maastricht V/Florence Consensus Report

What the new findings are:

- RT-PCR, which is not affected by previous PPI usage, can lead to a higher detection rate due to a higher sensitivity.
- In view of this high antibiotic resistance, testing for other antibiotic resistance needs to be also implemented. This will avoid treatment failures, unnecessary antibiotic prescription and thus a more targeted approach.
- RUT should not be the sole method of identification for the detection of *H. pylori* as was the procedure. Detection should be based on 2 or more methods of detection to avoid false positive or negative results.

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