

WJG 20<sup>th</sup> Anniversary Special Issues (6): *Helicobacter pylori***Antimicrobial susceptibility testing for *Helicobacter pylori* in times of increasing antibiotic resistance**

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Author contributions: Smith SM reviewed the literature, drafted and wrote the manuscript; O'Morain C and McNamara D critically reviewed the manuscript and provided intellectual input; all authors approved the manuscript for publication.

Supported by The Meath Foundation

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Received: October 17, 2013 Revised: February 13, 2014

Accepted: April 27, 2014

Published online: August 7, 2014

**Abstract**

The gram-negative bacterium *Helicobacter pylori* (*H. pylori*) causes chronic gastritis, gastric and duodenal ulcers, gastric cancer and mucosa-associated lymphoid tissue lymphoma. Treatment is recommended in all symptomatic patients. The current treatment options for *H. pylori* infection are outlined in this review in light of the recent challenges in eradication success, largely due to the rapid emergence of antibiotic resistant strains of *H. pylori*. Antibiotic resistance is a constantly evolving process and numerous studies have shown that the prevalence of *H. pylori* antibiotic resistance varies significantly from country to country, and even between regions within the same country. In addition, recent data has shown that previous antibiotic use is associated with harbouring antibiotic resistant *H. pylori*. Local surveillance of antibiotic resistance is warranted

to guide clinicians in their choice of therapy. Antimicrobial resistance is assessed by *H. pylori* culture and antimicrobial susceptibility testing. Recently developed molecular tests offer an attractive alternative to culture and allow for the rapid molecular genetic identification of *H. pylori* and resistance-associated mutations directly from biopsy samples or bacterial culture material. Accumulating evidence indicates that surveillance of antimicrobial resistance by susceptibility testing is feasible and necessary to inform clinicians in their choice of therapy for management of *H. pylori* infection.

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**Key words:** *Helicobacter pylori*; Antibiotic resistance; Antimicrobial susceptibility testing; Polymerase chain reaction; Molecular test

**Core tip:** There has been a significant decrease in the success rate of empirical triple therapy to treat *Helicobacter pylori* (*H. pylori*) infection, largely due to a rapid increase in the prevalence of antibiotic resistant strains. Antibiotic resistance is a constantly evolving process and there are significant regional variations in *H. pylori* antibiotic resistance rates. As such, local surveillance of antibiotic resistance is warranted to guide clinicians in their therapeutic choice. Standard culture-based antimicrobial susceptibility testing and molecular methods provide key opportunities to tailor *H. pylori* treatment based on the detection of antibiotic resistant strains, thereby enhancing eradication rates and decreasing *H. pylori*-associated disease.

Smith SM, O'Morain C, McNamara D. Antimicrobial susceptibility testing for *Helicobacter pylori* in times of increasing antibiotic resistance. *World J Gastroenterol* 2014; 20(29): 9912-9921 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v20/i29/9912.htm> DOI: <http://dx.doi.org/10.3748/wjg.v20.i29.9912>

## INTRODUCTION

*Helicobacter pylori* (*H. pylori*) specifically colonizes the gastric epithelium and is the most common human bacterial infection worldwide, infecting approximately half of the world's population<sup>[1-3]</sup>. Infection is usually acquired in early childhood and persists for decades if left untreated<sup>[1,2]</sup>. Prevalence of *H. pylori* infection varies globally but increases with older age and with lower socioeconomic status<sup>[4,5]</sup>. The higher prevalence in older age groups likely reflects poorer childhood living conditions in previous decades<sup>[2]</sup>. Although most infected patients will not develop any clinically significant complications, *H. pylori* infection confers a 1%-10% risk of developing gastric or duodenal ulcers, a 0.1%-3% risk of developing gastric adenocarcinoma, and < 0.01% of developing mucosa-associated lymphoid tissue (MALT) lymphoma<sup>[2]</sup>. *H. pylori* has been designated a Class I carcinogen by the World Health Organization<sup>[6]</sup>. Disease risk in infected individuals varies greatly in different populations and is associated with host genotype and strain-specific bacterial factors.

Patients with uncomplicated dyspepsia are managed using a "test-and-treat" strategy. *H. pylori* infection is diagnosed using non-invasive methods, including the urea breath test (UBT), serologic tests and the stool antigen test<sup>[2,3]</sup>. The UBT involves ingesting <sup>13</sup>C-labelled urea. If present, the *H. pylori* enzyme urease converts the <sup>13</sup>C-labelled urea to labelled carbon dioxide, which is detected in a breath sample. The UBT is easy to perform and highly accurate with a specificity and sensitivity of 95%<sup>[7,8]</sup>. The stool antigen test detects *H. pylori* antigens in stool samples, with laboratory-based stool antigen tests displaying specificity and sensitivity of at least 95%<sup>[9-11]</sup>. Serologic tests detect IgG antibodies to *H. pylori*. There is significant variability in the accuracy of serology test kits for *H. pylori*<sup>[12]</sup>, but validated commercial kits with an accuracy of over 90% are available<sup>[13]</sup>.

Endoscopy is warranted for dyspeptic patients with accompanying alarm symptoms such as weight loss, persistent vomiting, gastrointestinal bleeding, abdominal mass or iron-deficient anaemia<sup>[2,3]</sup>. In addition, endoscopy is recommended for patients with new onset dyspepsia above the age of 45 (European guidelines<sup>[3,14]</sup>) or 55 (United States guidelines<sup>[15]</sup>). *H. pylori* infection can be detected in endoscopic gastric biopsy samples by several methods. The rapid urease-test for *Campylobacter*-like organisms (CLO) involves placing the biopsy specimen in a solution of urea and pH-sensitive dye. The pH change resulting from the *H. pylori* urease-mediated conversion of urea to ammonia results in a colour change indicative of infection<sup>[2]</sup>. The CLO test has a sensitivity of greater than 90% and a specificity of more than 95%<sup>[7]</sup>. Histology on the biopsy specimen by means of staining with hematoxylin and eosin or Giemsa will also detect the presence of *H. pylori* infection and the degree of inflammation<sup>[2,3]</sup>. Immunohistochemistry-based tests with improved sensitivity have also been developed for the visualisation of *H. pylori* in biopsy samples<sup>[16-18]</sup>, but they are not yet used routinely. Culturing of the bacteria from biopsy samples

is also possible and has the added advantage of allowing for antimicrobial susceptibility testing. Acid suppressing proton pump inhibitors (PPIs) should be avoided 2 wk prior to diagnostic testing by the UBT, stool antigen test or endoscopy as they increase the gastric pH, leading to a decrease in bacterial load, and may result in a false negative *H. pylori* diagnosis<sup>[3,19]</sup>. In addition, antimicrobials should be avoided for 4 wk prior to testing (UBT, stool antigen test or endoscopy) as these agents also suppress infection and reduce test sensitivity<sup>[2]</sup>. Serological tests that detect antibodies to *H. pylori* are the only methods that are not affected by decreased gastric bacterial load, which may lead to false negative results using the other diagnostic testing methods outlined above<sup>[3]</sup>.

## TREATMENT FOR *H. PYLORI* INFECTION

Treatment for *H. pylori* is recommended in all symptomatic individuals. Eradication of *H. pylori* infection provides a long-term cure for both duodenal and gastric ulcers in the majority of patients whose ulcers are not associated with non-steroidal anti-inflammatory drug use<sup>[20,21]</sup>. In addition, evidence suggests that *H. pylori* eradication reduces the development of atrophic gastritis and the risk of cancer progression in infected individuals without premalignant gastric lesions<sup>[22-29]</sup>. Furthermore, eradication of infection leads to regression of most localized gastric MALT lymphomas<sup>[30-33]</sup>.

The standard empirical triple therapy for *H. pylori* proposed at the first Maastricht conference on the management of *H. pylori* infection<sup>[34]</sup> has become widely used throughout the world<sup>[15,35,36]</sup>. This first-line therapy consists of a PPI with the antibiotics clarithromycin and amoxicillin taken twice daily for 7-14 d<sup>[3]</sup>. Metronidazole is used instead of amoxicillin in patients with a penicillin allergy<sup>[2]</sup>. The success rate of first-line treatment has fallen below the recommended 80% in recent years<sup>[3,5]</sup>. Non-compliance<sup>[37]</sup> and the emergence of antibiotic resistant strains of *H. pylori*<sup>[38-42]</sup> are considered to be the major factors contributing to treatment failure. Standard empirical triple therapy is now only recommended in regions where clarithromycin resistance is known to be less than 15%-20%<sup>[3]</sup>. While no new treatment has been developed as an alternative to the standard triple therapy, recent studies have described the advantages of using different combinations of known antibiotics or extended treatment durations. In regions where clarithromycin resistance is greater than 15%-20%, bismuth quadruple therapy consisting of a PPI, a bismuth salt, tetracycline and metronidazole is recommended<sup>[3]</sup>. As bismuth salts are not available in every country, non-bismuth quadruple therapies, namely sequential therapy (5 d PPI and amoxicillin; 5 d PPI with clarithromycin and metronidazole) or concomitant therapy (PPI with amoxicillin, metronidazole and clarithromycin) may be prescribed as alternative therapies<sup>[3]</sup>. Following treatment, eradication of *H. pylori* should be confirmed by the UBT, stool antigen test or by endoscopy if required. As antibodies persist

for months following infection, serology testing is not recommended for eradication confirmation<sup>[2]</sup>. Following failure of initial therapy, a PPI with amoxicillin and levofloxacin is recommended. As non-compliance may lead to treatment failure, adherence should be strongly emphasised for subsequent therapies. If third-line treatment is required, a number of studies support quinolone or rifabutin-based regimens<sup>[3,43,44]</sup> but treatment should only be prescribed following antimicrobial susceptibility testing where possible<sup>[3]</sup>.

### ***H. pylori* culture and antimicrobial susceptibility testing**

*H. pylori* culture and antimicrobial susceptibility testing is carried out in an effort to predict antibiotic treatment outcome and guide clinicians in their choice of therapy. Culture and antimicrobial susceptibility testing guidelines have been outlined by the European *Helicobacter* Study Group (EHSG)<sup>[42]</sup>. As use of PPIs or antimicrobials inhibits the growth of *H. pylori* and reduces the chances of successful culture, patients should avoid taking PPIs for at least 2 wk and antimicrobials for 4 wk prior to endoscopy<sup>[3,19]</sup>. Biopsy specimens should be transported and processed for culture as soon as possible, ideally within 6 h<sup>[45]</sup>. If processing is delayed refrigeration is recommended<sup>[45,46]</sup>. Biopsy specimens are used to inoculate Columbia blood agar plates containing 10% laked horse blood and incubated under microaerophilic conditions at 37 °C for 7-10 d<sup>[19]</sup>, although colonies are usually visible at 3-5 d<sup>[45]</sup>. Antimicrobial supplements may be added to media to inhibit overgrowth with contaminating bacteria and fungi<sup>[19,47]</sup>. The presence of *H. pylori* should be confirmed by the Gram stain, and positive oxidase, urease and catalase tests. Fresh cultures (48-72 h growth) at an inoculum concentration of McFarland 3<sup>[19]</sup> should be used for *H. pylori* culture-based antimicrobial susceptibility testing<sup>[45]</sup>. Culture medium manufactured specifically for antimicrobial susceptibility testing (e.g., Mueller Hinton agar; Oxoid, Basingstoke, United Kingdom)<sup>[19]</sup> should be used and the depth of the agar should be kept consistent across tests.

Several assays are commercially available to test the antimicrobials commonly used to treat *H. pylori*. The disc diffusion method (Oxoid) involves placing an antibiotic-coated disc directly onto the agar plate inoculated with *H. pylori* and determining the zone of bacterial growth inhibition. This cost-effective method is widely used for antimicrobial susceptibility testing for a variety of microorganisms, but disc diffusion criteria for antimicrobial susceptibility testing for *H. pylori* have not to date been defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST)<sup>[48]</sup>. Both EUCAST and the British Society for Antimicrobial Chemotherapy (<http://bsac.org.uk>) recommend Etest strips for *H. pylori* culture-based antimicrobial susceptibility testing. Etests (Biomerieux; Basingstoke, United Kingdom) are plastic strips calibrated with a predefined concentration gradient of antibiotic and enable the quantitative determination of the minimum inhibitory concentration (MIC) of an

**Table 1 Proposed clinical antimicrobial breakpoints for *Helicobacter pylori***

Antibiotic	Susceptible (mg/L)	Resistant (mg/L)
Amoxicillin	≤ 0.12	> 0.12
Clarithromycin	≤ 0.25	> 0.5
Metronidazole	≤ 8	> 8
Levofloxacin	≤ 1	> 1
Rifampicin <sup>1</sup>	≤ 1	> 1
Tetracycline	≤ 1	> 1

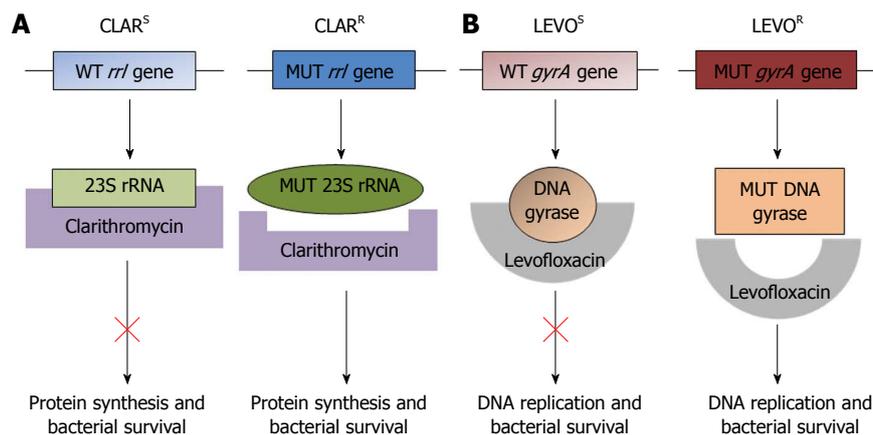
<sup>1</sup>Although rifabutin is used clinically, rifabutin E tests are not available routinely and rifampicin is used to screen for rifabutin resistance. Adapted from European Committee on Antimicrobial Susceptibility Testing<sup>[48]</sup>.

antimicrobial agent required to inhibit bacterial growth. The MIC can be read directly from the scale printed on the strip at the point where the edge of the inhibition ellipse of bacterial culture intersects with the strip. The EUCAST breakpoints for antimicrobial resistance to *H. pylori* are listed in Table 1. Etest MIC results may be validated subsequently using agar dilution approaches. When performing antimicrobial susceptibility testing, quality control tests involving reference strains with known susceptibility or resistance should be included.

## **MOLECULAR TESTING FOR ANTIBIOTIC RESISTANCE-ASSOCIATED MUTATIONS**

While *H. pylori* culture allows an evaluation of antibiotic resistance irrespective of the intrinsic mechanism involved, *H. pylori* is a fastidious bacterium and culture is time-consuming and often difficult with sensitivity values of culture from gastric biopsies as low as 55%-73%<sup>[49-52]</sup>. Molecular testing for *H. pylori* offers an attractive alternative to culture and allows for molecular genetic identification of *H. pylori* directly from biopsy samples in addition to culture material. As such, it provides the opportunity for rapid analysis, enabling same-day diagnosis. Molecular testing has been recommended to detect *H. pylori* and both clarithromycin and quinolone resistance when standard culture and susceptibility testing are not possible<sup>[3]</sup>.

The genetic mutations conferring resistance to clarithromycin and quinolones have been well characterised<sup>[53]</sup>. Clarithromycin functions by binding and suppressing the activity of the bacterial ribosomal subunit, thus inhibiting protein synthesis<sup>[4]</sup> (Figure 1). Single point mutations within the *H. pylori rrl* gene encoding the 23S ribosomal RNA component of ribosomes result in clarithromycin resistance. The three most frequently occurring mutations, A2146C, A2146G and A2147G (Genbank Accession number NC\_000915; formerly described as A2142C, A2142G and A2143G), are thought to account for 90% of primary clarithromycin resistance in Western countries<sup>[4,53-57]</sup>. These mutations can be detected by a number of polymerase chain reaction (PCR)-based molecular methods using both bacterial culture samples<sup>[54,58,59]</sup> and gastric biopsies<sup>[55,59-61]</sup>. In addition, these assays have been used to analyse stool samples<sup>[62-65]</sup>, providing an opportu-



**Figure 1** Molecular mechanism of clarithromycin and levofloxacin resistance. A: Clarithromycin binds and suppresses the activity of the bacterial ribosomal subunit, thus inhibiting protein synthesis. Single point mutations within the *Helicobacter pylori* (*H. pylori*) *rrl* gene encoding the 23S ribosomal RNA component of ribosomes result in clarithromycin resistance. The best characterised point mutations conferring clarithromycin resistance are A2146C, A2146G and A2147G; B: Levofloxacin targets *H. pylori* DNA gyrase. The most significant mutations conferring levofloxacin resistance occur at positions 87 and 91 of the *H. pylori* *gyrA* gene, which encodes the A subunit of the DNA gyrase enzyme involved in regulating the topological state of bacterial DNA during replication. CLAR<sup>S</sup>: Clarithromycin sensitive; CLAR<sup>R</sup>: Clarithromycin resistant; LEVO<sup>S</sup>: Levofloxacin sensitive; LEVO<sup>R</sup>: Levofloxacin resistant; WT: Wild type; MUT: Mutant.

**Table 2** Sensitivity and specificity of molecular tests for detecting *Helicobacter pylori* antibiotic resistance

Molecular test	Antibiotic	Sensitivity	Specificity	Ref.
PCR	Clarithromycin	82%	100%	[64]
Genotype	Clarithromycin	94%	99%	[57]
HelicoDR assay	Levofloxacin	87%	98.5%	
PCR	Clarithromycin	90.6%	95.8%	[59]
PCR	Clarithromycin	89.2%	100%	[62]
Genotype	Clarithromycin	100%	86.2%	[72]
HelicoDR assay	Levofloxacin	82.6%	95.1%	
PCR	Clarithromycin	83.3%	100%	[65]

PCR: Polymerase chain reaction.

nity for diagnosis of *H. pylori* infection and antimicrobial susceptibility testing through non-invasive procedures. Several molecular testing kits are commercially available for the detection of clarithromycin resistance, including the MutaREAL *H. pylori* kit<sup>[59]</sup> (Immunodiagnostik; Benshiem, Germany), the ClariRes real-time PCR assay<sup>[63]</sup> (Ingentix; Vienna Austria) and the Seeplex ClaR-*H. pylori* ACE detection system<sup>[61]</sup> (Seegene; Eschborn, Germany). Accumulating evidence has demonstrated that the presence of mutations detected by molecular tests correlates well with culture-based susceptibility testing<sup>[54,60,61]</sup>.

The most significant mutations conferring quinolone resistance lie at positions 87 and 91 of the *H. pylori* *gyrA* gene, which encodes the A subunit of the DNA gyrase enzyme involved in regulating the topological state of bacterial DNA during replication (Figure 1)<sup>[53,57,66,67]</sup>. PCR-based molecular assays have successfully detected quinolone resistant strains of *H. pylori*<sup>[59,66,67]</sup>. The mechanism of resistance to metronidazole is less clear. Mutations in the *rdxA* and *frxA* genes have been implicated; however, a definitive panel of point mutations accounting for metronidazole resistance has not yet been described<sup>[4]</sup>. Although mutations in the DNA encoding the 16S rRNA

involved in tetracycline resistance, the *rpoB* gene involved in rifabutin resistance and the *pbp-1a* gene involved in amoxicillin resistance have also been described<sup>[4,46,68-70]</sup>, they are of less interest since the rates of *H. pylori* resistance to these antibiotics are low in most regions<sup>[19,42,70,71]</sup>.

The recently developed GenoType HelicoDR assay (Hain Lifescience; Nehren, Germany) enables determination of resistance to clarithromycin and quinolones through a protocol involving DNA extraction from either bacterial cultures or biopsy material, multiplex PCR amplification of *H. pylori* DNA sequences and hybridization against probes that detect specific *H. pylori* wild-type or mutated elements. The GenoType HelicoDR assay is highly accurate with a sensitivity and specificity of 94%-100% and 86%-99% respectively for clarithromycin resistance and 83%-87% and 95%-98.5% respectively for quinolone resistance<sup>[57,72]</sup>. Studies defining the sensitivity and specificity of molecular tests for detecting *H. pylori* clarithromycin and levofloxacin resistance are summarized in Table 2.

Several issues are to be considered when choosing a molecular assay for *H. pylori* antimicrobial susceptibility testing, including cost, local expertise in molecular diagnosis, the equipment available and the sensitivity and specificity of the test<sup>[53]</sup>. Molecular-based techniques are highly accurate in detecting minimal traces of antibiotic resistant *H. pylori* strains, even in small tissue samples. Moreover these tools are accurate in detecting the co-existence of *H. pylori* strains susceptible and resistant to the same antibiotic within the same patient sample, known as hetero-resistance<sup>[4,55,73]</sup>. This is potentially important given that in one recent study molecular-based tests indicated that in relation to genetic clarithromycin resistance, *H. pylori* infection was cured less frequently in patients with pure resistant strains (46%) than those infected with hetero-resistant strains (78.5%) or susceptible strains (94.5%)<sup>[55]</sup>.

**Table 3** Summary of Europe-wide studies on antibiotic resistance rates over time

Antibiotic	1991 <sup>[93]</sup>	1998 <sup>[74]</sup>	2008/9 <sup>[42]</sup>
Metronidazole	27.5%	33.1%	34.9%
Clarithromycin	ND	9.9%	17.5%
Amoxicillin	ND	0.8%	0.7%
Levofloxacin	ND	ND	14.1%
Tetracycline	ND	ND	0.9%
Rifampicin	ND	ND	1.1%

ND: No data.

It must be kept in mind that the majority of molecular tests available do not detect resistance based on uncommon genetic mechanisms. Additional rare mutations within the *rrl* and *gyrA* gene regions conferring clarithromycin and quinolone resistance respectively have been described<sup>[59,60]</sup>. In addition, resistances of *H. pylori* strains that lie outside the *rrl* and *gyrA* genes, as well as other possible mechanisms of clarithromycin and quinolone resistance must be considered. Further studies are required to determine if molecular tests predict *H. pylori* treatment failure more accurately than phenotypic culture and sensitivity tests.

## H. PYLORI ANTIMICROBIAL RESISTANCE

Most patients are prescribed initial *H. pylori* eradication treatment without culture and antibiotic susceptibility testing as current guidance recommend a “test-and-treat” strategy based on non-invasive diagnostic methods<sup>[3,15,35]</sup>. As the most recent Maastricht IV consensus guidelines recommend that clarithromycin should not be used to treat *H. pylori* if resistance rates are above 15%-20%<sup>[3]</sup>, surveillance of primary antibiotic resistance is warranted to guide clinicians in their choice of therapy. The EHSG have recently reported on the prevalence of primary *H. pylori* antibiotic resistance from 2008-2009 using a multicentre approach with standardised protocols<sup>[42]</sup>. The number of test centres involved in the study was proportional to population of each country, with 2204 patients included from 32 centres in 18 European countries. The overall primary resistance rates for clarithromycin, levofloxacin and metronidazole were 17.5%, 14.1% and 34.9% respectively, with a prevalence  $\leq$  1% for tetracycline, rifampicin and amoxicillin<sup>[42]</sup>. Combined resistance to metronidazole and clarithromycin was found in 7.8% of strains. The rate of clarithromycin resistance had almost doubled since the previous European survey<sup>[74]</sup> (Table 3), which has important implications considering that clarithromycin resistance decreases the efficacy of clarithromycin-amoxicillin-PPI triple therapy by up to 70%<sup>[75-77]</sup>. Prevalence of levofloxacin resistance was not tested in the previous European study<sup>[74]</sup> as levofloxacin-based treatment was introduced later, but several studies have shown an emergence in levofloxacin resistance in the last decade<sup>[42,67,78]</sup>. Metronidazole resistance was high at 34.9%<sup>[42]</sup> but the rate was similar to that of the previ-

**Table 4** Recent data on *Helicobacter pylori* antibiotic resistance rates in different regions and countries

Ref.	Region	Antibiotic resistance rates		
		Clar	Met	Levo
Tveit <i>et al</i> <sup>[94]</sup>	Alaska	30% <sup>2</sup>	42% <sup>2</sup>	19% <sup>2</sup>
Gao <i>et al</i> <sup>[71]</sup>	China; Beijing	37.2% <sup>2</sup>	63.9% <sup>2</sup>	50.3% <sup>2</sup>
Su <i>et al</i> <sup>[83]</sup>	China; South East coastal region	21.5% <sup>2</sup>	95.4% <sup>2</sup>	20.6% <sup>2</sup>
McNulty <i>et al</i> <sup>[19]</sup>	England	3% <sup>2</sup>	22% <sup>2</sup>	1% <sup>2</sup>
Megraud <i>et al</i> <sup>[42]</sup>	Europe; Northern countries	7.7% <sup>1</sup>	28.6% <sup>1</sup>	7.7% <sup>1</sup>
Megraud <i>et al</i> <sup>[42]</sup>	Europe; Southern countries	21.5% <sup>1</sup>	29.7% <sup>1</sup>	13.1% <sup>1</sup>
Megraud <i>et al</i> <sup>[42]</sup>	Europe; Western and central countries	18.7% <sup>1</sup>	43.8% <sup>1</sup>	18.6% <sup>1</sup>
Abadi <i>et al</i> <sup>[95]</sup>	Iran	45.2% <sup>2</sup>	65.5% <sup>2</sup>	34.5% <sup>2</sup>
O'Connor <i>et al</i> <sup>[81]</sup>	Ireland	9.3% <sup>1</sup>	29.1% <sup>1</sup>	ND
O'Connor <i>et al</i> <sup>[82]</sup>		13.2% <sup>2</sup>	31.5% <sup>2</sup>	11.7% <sup>2</sup>
Yamada <i>et al</i> <sup>[84]</sup>	Japan	38.8% <sup>1</sup>	ND	34% <sup>1</sup>
		55.6% <sup>2</sup>	ND	38.6% <sup>2</sup>
Lee <i>et al</i> <sup>[85]</sup>	Korea	23.7% <sup>1</sup>	ND	28.1% <sup>1</sup>
Seck <i>et al</i> <sup>[96]</sup>	Senegal	1% <sup>1</sup>	85% <sup>1</sup>	15% <sup>1</sup>
Vilachione <i>et al</i> <sup>[70]</sup>	Thailand	3.7% <sup>1</sup>	36% <sup>1</sup>	7.2% <sup>1</sup>
McNulty <i>et al</i> <sup>[19]</sup>	Wales	18% <sup>2</sup>	43% <sup>2</sup>	13% <sup>2</sup>

<sup>1</sup>Primary antibiotic resistance rates; <sup>2</sup>Overall resistance rates. Clar: Clarithromycin; Met: Metronidazole; Levo: Levofloxacin; ND: No data presented in this study.

ous Europe-wide study<sup>[74]</sup> (Table 3). The impact of metronidazole resistance on *H. pylori* eradication is less than that of clarithromycin resistance, and can be overcome by increasing the dose and duration of treatment or by prescription of bismuth-containing quadruple therapy that includes metronidazole<sup>[38,79,80]</sup>. The latest EHSG study on antibiotic resistance also indicated variations across European countries; the resistance rate for clarithromycin was < 10% in Northern European countries, while most countries in the rest of Europe (except Spain and Germany) had a resistance rate of > 15% (Table 4)<sup>[42]</sup>. Such variations in antibiotic resistance have also been reported on a regional basis. For example, a recent study within the United Kingdom indicated that the resistance rates to clarithromycin, metronidazole and quinolones were 18%, 43%, 13% respectively at a test centre in Wales but the rates were lower in an English test centre at 3%, 22%, 1%<sup>[19]</sup>. Recent findings from Irish patient cohorts indicate that the rates of clarithromycin, metronidazole and levofloxacin were 9.3%, 29.1%<sup>[81]</sup> and 11.7% respectively<sup>[82]</sup>.

Variations in resistance rates have also been reported outside Europe (Table 4)<sup>[4,5]</sup>. In Thailand for example, the resistance rates for clarithromycin, metronidazole and levofloxacin are 3.7%, 36% and 7.2% respectively, with significantly higher rates of metronidazole resistance in Southern Thailand than North Eastern Thailand (66.7% *vs* 33.3%)<sup>[70]</sup>. In the South East coastal region of China resistance rates for clarithromycin, metronidazole and levofloxacin are 21.5%, 95.4% and 20.6%<sup>[83]</sup> respectively, while in Beijing the rates are 37.2%, 63.9% and 50.3% for clarithromycin, metronidazole and levofloxacin re-

spectively<sup>[71]</sup>. A recent study from Japan indicates that the prevalence of clarithromycin resistance is 55.6% and levofloxacin resistance is 38.6%<sup>[84]</sup>, while in South Korea, an increase in the resistance rates for clarithromycin (17.2%-23.7%) and quinolones (4.7%-28.1%) was reported from 2003 to 2012<sup>[85]</sup>.

Emergence of antibiotic resistance is thought to be associated with prior antibiotic use. Indeed, by analysing cumulative and yearly outpatient antibiotic consumption the pan-European study demonstrated a significant association between the use of long acting macrolides and resistance of *H. pylori* to clarithromycin, and between previous quinolone use and levofloxacin resistance<sup>[42]</sup>. Using an alternative approach to analyse data from GP and patient records, the United Kingdom study demonstrated that each previous course of clarithromycin, metronidazole or quinolone taken by an individual was associated with an increase in the risk harbouring an antibiotic resistant strain of *H. pylori*<sup>[19]</sup>.

## CONCLUSION

Consensus guidelines recommend abandoning clarithromycin in empirical triple therapy when the prevalence of clarithromycin resistance is higher than 15%-20%<sup>[3]</sup>. This level has now been reached in most countries in Western/Central and Southern Europe<sup>[42]</sup> as well as many countries in Asia<sup>[71,83-85]</sup>. Information on resistance rates is not widely available to clinicians, especially at a local level. As antibiotic resistance is a constantly evolving process and there is significant variation in resistance rates between countries and within different regions of the same country<sup>[19,70,71,83]</sup>, it is important that local surveillance of primary antibiotic resistance is performed regularly and that anti-*H. pylori* treatment regimens should be chosen according to local resistance data. Increasing resistance rates for the second-line antibiotic levofloxacin have been reported in several countries<sup>[42,71,83,84]</sup>. Culture and antimicrobial susceptibility testing should be considered in all regions before second-line treatment is prescribed if endoscopy is performed<sup>[3]</sup>. Furthermore, antimicrobial susceptibility testing is recommended in all regions when a second-line treatment has failed<sup>[3]</sup>. If standard culture and susceptibility testing is not possible, molecular tests can be used to detect antibiotic resistance. The recent studies by McNulty *et al.*<sup>[19]</sup> and the EHSG<sup>[42]</sup> indicate that on-going surveillance of antimicrobial resistance is indeed feasible and necessary to inform clinicians in the management of *H. pylori* infection. Alternatively, as previous antibiotic use is linked to an increased risk of antibiotic resistance<sup>[19,42]</sup>, knowledge of previous antibiotic consumption may provide a tool to predict the susceptibility of *H. pylori* to antimicrobial agents and adapt treatment strategies where culture and susceptibility testing or molecular testing are not available.

Evidence from numerous studies provides a rationale for tailoring treatment based on antimicrobial susceptibility testing to improve eradication rates for primary and sub-

sequent anti-*H. pylori* treatment regimens. A recent meta-analysis by Wenzhen *et al.*<sup>[86]</sup> of five randomised control trials totalling 701 patients has shown that tailored treatments based on resistance data show better eradication rates than standard empirical triple therapy. Tailored triple therapy based on culture and sensitivity testing was also found to be more cost effective than standard triple therapy for first line treatment in a study by Romano *et al.*<sup>[87]</sup>. However, others argue against the cost-effectiveness of culture-based treatment for first line therapy<sup>[88,89]</sup>. The economic benefits of tailoring first line therapy are likely to depend on the local antibiotic resistance levels as a recent study by Cosme *et al.*<sup>[90]</sup> reported that performing culture and antimicrobial susceptibility testing lead to higher eradication rates and increased cost efficiency in an area where clarithromycin resistance was high (> 15%-20%).

With regard to tailoring rescue regimens for anti-*H. pylori* treatment, Fiorini *et al.*<sup>[91]</sup> demonstrated that culture-based therapy eradicated *H. pylori* infection in 90% of patients who had not previously responded to treatment. Liou *et al.*<sup>[92]</sup> showed increased efficacy of sequential therapy guided by PCR-based molecular tests in the third-line treatment of refractory *H. pylori* infection. Furthermore, newly developed methods allow the detection of *H. pylori* and antibiotic resistance in stool samples<sup>[62-65]</sup>, providing a potential opportunity to assess the prevalence of antimicrobial resistance by non-invasive methods without the expense of endoscopy.

It is clear that the emerging rates of antimicrobial resistance represent a significant challenge in the successful management of *H. pylori* infection and that resistance surveillance is warranted. The recent evidence highlights the importance of antimicrobial susceptibility testing in both the on-going assessment of primary antibiotic resistance rates and in tailoring treatments to increase *H. pylori* eradication success. In addition to standard culture-based antimicrobial susceptibility testing, molecular methods provide key opportunities in detecting antibiotic resistant strains of *H. pylori*, enhancing eradication rates and decreasing *H. pylori*-associated disease.

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**P- Reviewer:** Borgmann S, Kelesidis T, Mastroianni CM, Wang JT

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ISSN 1007-9327

