AP&T Alimentary Pharmacology and Therapeutics

Review article: the global emergence of *Helicobacter pylori* antibiotic resistance

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Publication data
Submitted 7 April 2015
First decision 4 May 2015
Resubmitted 15 October 2015
Resubmitted 18 November 2015
Accepted 19 November 2015
EV Pub Online 23 December 2015

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This uncommissioned review article was subject to full peer-review.

SUMMARY

Background
Helicobacter pylori is one of the most prevalent global pathogens and can lead to gastrointestinal disease including peptic ulcers, gastric marginal zone lymphoma and gastric carcinoma.

Aim
To review recent trends in *H. pylori* antibiotic resistance rates, and to discuss diagnostics and treatment paradigms.

Methods
A PubMed literature search using the following keywords: *Helicobacter pylori*, antibiotic resistance, clarithromycin, levofloxacin, metronidazole, prevalence, susceptibility testing.

Results
The prevalence of bacterial antibiotic resistance is regionally variable and appears to be markedly increasing with time in many countries. Concordantly, the antimicrobial eradication rate of *H. pylori* has been declining globally. In particular, clarithromycin resistance has been rapidly increasing in many countries over the past decade, with rates as high as approximately 30% in Japan and Italy, 50% in China and 40% in Turkey; whereas resistance rates are much lower in Sweden and Taiwan, at approximately 15%; there are limited data in the USA. Other antibiotics show similar trends, although less pronounced.

Conclusions
Since the choice of empiric therapies should be predicated on accurate information regarding antibiotic resistance rates, there is a critical need for determination of current rates at a local scale, and perhaps in individual patients. Such information would not only guide selection of appropriate empiric antibiotic therapy but also inform the development of better methods to identify *H. pylori* antibiotic resistance at diagnosis. Patient-specific tailoring of effective antibiotic treatment strategies may lead to reduced treatment failures and less antibiotic resistance.

Aliment Pharmacol Ther 2016; **43:** 514–533

doi:10.1111/apt.13497

INTRODUCTION

Helicobacter pylori is one of the most prevalent global pathogens and colonises an estimated 50% of the world's population.[1, 2] It was first described in gastric biopsies by Warren and Marshall in Australia in 1983.[3–5] *H. pylori* is a Gram-negative bacillus that infects the human stomach mucosa and produces diseases of the upper gastrointestinal tract such as chronic gastritis, peptic ulcer disease, gastric marginal zone/mucosa-associated lymphoid tissue (MALT) lymphoma and gastric carcinoma.[3, 4, 6, 7] More recently, it has been suggested that *H. pylori* may be associated with extraintestinal diseases, including immune thrombocytopenic purpura, refractory iron deficiency anaemia and vitamin B12 deficiency.[1, 8, 9]

The precise epidemiology of *H. pylori* infection still remains unclear; however, studies have shown that ingestion of contaminated food may increase the risk of *H. pylori* infection. Person-to-person transmission by oral-oral, faecal-oral or gastro-oral exposure is suggested to be the most likely route of transmission.[10] Accordingly, improvements in hygiene and living conditions are important factors in decreasing the prevalence of infection.[11] In addition, mammal and insect reservoirs have been suggested such as pigtailed monkeys, rhesus monkeys, cats, sheep and cockroaches.[12] There have also been association studies with maternal infection and socioeconomic status being an important risk factor for paediatric infection.[13–17]

Current treatment of *H. pylori* in the United States is empiric despite relatively high failure rates in more than 20% of cases.[18] In the early 1990s the eradication rate of *H. pylori* was greater than 80%.[19–21] However, the antimicrobial eradication rates are decreasing – as low as 60% in some countries – and are inversely correlated with antibiotic resistance rates reported worldwide.[19, 20] Eradication failures are concerning at the present time, but they are likely to be more critical in the future, given that global rates of antibiotic resistance are increasing and therapy for *H. pylori* infection is increasingly prescribed.[21–24] This is reflected in the most recent Maastricht recommendations which state that susceptibility testing should be performed prior to therapy in regions with high clarithromycin resistance rates.[1] According to the recent Kyoto Global Consensus Meeting, only regimens shown to result in at least a 90% eradication rate in a particular region should be used as empiric therapy.[25] Therefore, the goal in designing a treatment regimen should focus on a strategy which results in a cure rate approaching 100%.[21, 26–28]

PREVALENCE OF *H. PYLORI* INFECTION

Infection with *H. pylori* occurs worldwide, but there are substantial geographic differences in the prevalence of infection both within and between countries.[29] Multiple studies have demonstrated that low socioeconomic status is associated with increased risk of *H. pylori* infection.[30, 31] Additionally, an age-related cohort effect has been observed with prevalence of infection increasing with age.[31–33] Within Europe, *H. pylori* prevalence rates range from 11% in Sweden to 60.3% in Spain.[29, 34] In China, *H. pylori* prevalence has been reported as high as 83.4%.[35] Additionally, many countries such as China, Japan and Bulgaria have experienced an overall increase in the prevalence of *H. pylori* infection over the last 20 years (Figure 1).[29, 34–53] In Canada, the prevalence of *H. pylori* is approximately 30%; however, within the Aboriginal populations living in Canada, the prevalence of *H. pylori* has been reported as high as 95%.[54, 55] In the USA, cross-sectional studies of the participants in the National Health and Nutrition Examination Survey (NHANES) III and NHANES 1999–2000 demonstrate an overall seropositivity rate of approximately 30%.[29, 56] In populations with high infection rates, it is likely that patients are infected with more than one strain of *H. pylori*.

PREVALENCE OF ANTIBIOTIC RESISTANCE

The prevalence of bacterial resistance varies in different geographic areas and appears to be increasing with time in many countries.[57, 58] While the overall prevalence of *H. pylori* in the USA has been similar in studies from both 2000 and 2010, the antimicrobial eradication rates for *H. pylori* have been decreasing over that interval for several reasons; the most likely primary reasons for treatment failure were found to be *H. pylori* resistance to one or more of the antibiotics and patient compliance.[1, 23, 29, 53, 59–61]

The European Multicentre Study Group included 2204 patients from 2008 to 2009, spanning 18 European countries and demonstrated *H. pylori* resistance rates to clarithromycin, metronidazole and levofloxacin at 17.5%, 34.9% and 14.1% respectively.[22] The Japanese National Surveillance Study looked at 3707 *H. pylori* isolates from 2002 to 2005. Kobayashi *et al.* found that clarithromycin resistance rates increased from 18.9% to 27.7% between this 3-year interval. Metronidazole resistance remained fairly consistent, ranging from 3.3% to 5.3%. Amoxicillin resistance rates were negligible.[62] The Surveillance of *H. pylori* Antimicrobial Resistance Partnership (SHARP) program meta-analysis conducted between 1993 and

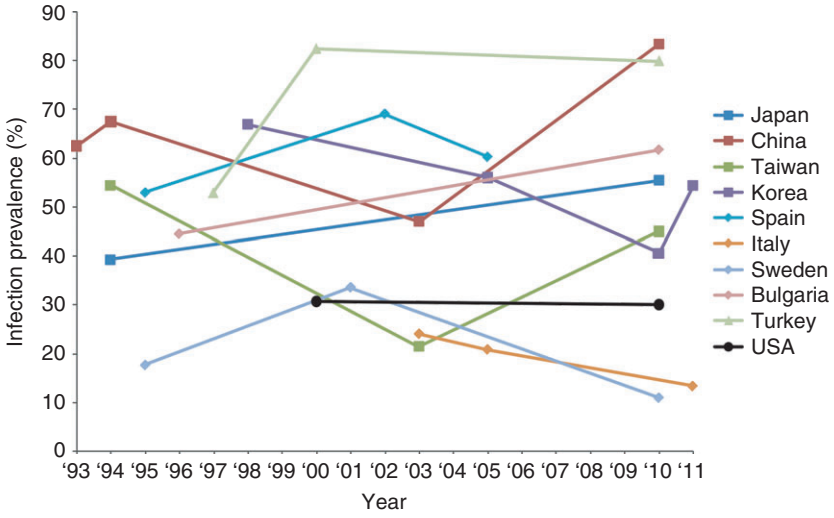

Figure 1 | Global prevalence of *H. pylori* infection by country and year.[29, 34–53]

1999 demonstrated that the resistance rates to clarithromycin, metronidazole and amoxicillin are 10.1%, 36.9% and 1.4% respectively.[57] The prospective multicenter *Helicobacter pylori* Antimicrobial Resistance Monitoring Program (HARP) study examined the national incidence rates of *H. pylori* antimicrobial resistance between 1998 and 2002; this study demonstrated the resistance to clarithromycin, metronidazole and amoxicillin as 12.9%, 25.1% and 0.9% respectively.[63]

There is evidence that bacterial resistance is correlated with the general consumption of antibiotics in the general population.[22, 64] In a study focused on the Alaskan Native population, prior use of a macrolide was associated with increased *H. pylori* clarithromycin resistance. Overall, 37% of this Alaskan Native population was infected with clarithromycin-resistant strains of *H. pylori* and, of these patients, 92% had previously been treated with a macrolide. Additionally, McMahon *et al.* found a dose–response relationship between clarithromycin resistance and the number of prior macrolide courses. Within this population, a similar association was found with infection by metronidazole-resistant *H. pylori* strains and prior metronidazole use.[65–67] Evaluating and understanding this relationship between previous antimicrobial use and *H. pylori* antimicrobial resistance would help to increase cure rates.[68, 69]

Clarithromycin

Numerous studies have been performed to determine the prevalence of *H. pylori* resistance to particular antibiotics. In particular, clarithromycin-resistant *H. pylori* has been extensively studied. The prevalence has been increasing in many countries (Figure 2a).[22, 48, 70–85] Studies have shown that in countries with low rates of *H. pylori* seropositivity, the prevalence of antibiotic resistance does not appear to change considerably over time. For instance, *H. pylori* seropositivity rates in Sweden have remained approximately 20% since 1995.[29, 48, 49] The prevalence of clarithromycin-resistant strains of *H. pylori* has also remained low (below 5%) over this period of time.[48, 74, 79] In contrast, countries with higher rates of *H. pylori* seropositivity are associated with dramatically increasing rates of clarithromycin resistance. For example, Horiki *et al.* demonstrated that the prevalence of clarithromycin resistance has increased considerably from 1.8% in 1996 to 27.1% in 2008 in the Japanese population.[70] Okamura *et al.* described an overall resistance rate of 31.1% in patients studied between 2000 and 2013.[71] The prevalence of *H. pylori* seropositivity over this time period has increased from approximately 40% to 55%.[29,36,70] Similarly, China has experienced an increase in clarithromycin resistance from 14.8% in 2000 to 52.6% in 2014 with an increase in seropositivity rates from approximately 65% to 83%.[35, 37, 38, 72–74] In addition, a marked increase in prevalence of clarithromycin resistance was seen in Korea from 11% in 2005 to 60% in 2009.[76–78] The variability of clarithromycin resistance seen in different regions emphasises the need to examine resistance rates in each geographic area to better guide treatment regimens. Within the USA, the prevalence of clarithromycin resistance has increased from 6.1% in 1993 to 12.9% in 2002.[58, 61, 84] A recent report studying a population of military veterans from the USA between 2009 and 2013 demonstrated an overall 17.8% clarithromycin resistance rate.[85] Within the paediatric population, the rate of clarithromycin resistance has been reported to be as high as 50%.[86] The dramatic increase in clarithromycin resistance observed in recent reports from the USA suggests the need for an updated national survey.

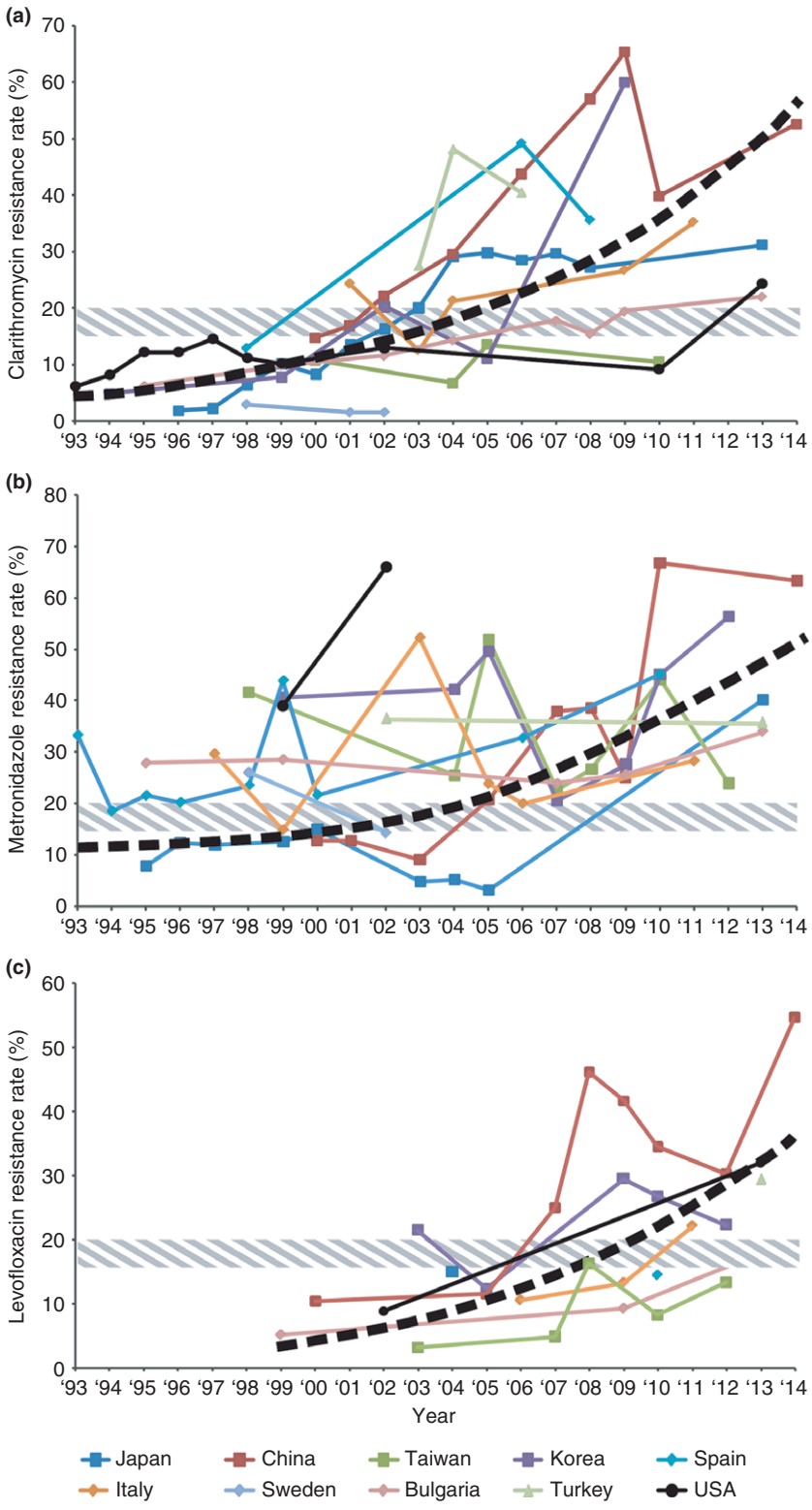

Figure 2 | (a) Global prevalence of clarithromycin antibiotic resistance by country and year.[22, 48, 70–85] (b) Global prevalence of metronidazole antibiotic resistance by country and year.[62, 65, 71–77, 79, 81–84, 87–106] (c) Global prevalence of levofloxacin antibiotic resistance by country and year.[22, 67, 72–74, 77, 81–83, 85, 87, 88, 90, 95, 96, 106, 117–121] Hashed band indicates threshold for altering therapeutic intervention.

Metronidazole

Overall, metronidazole resistance rates have been increasing in many countries (Figure 2b).[62, 65, 71–77, 79, 81–84, 87–106] The prevalence of *H. pylori* resistance to metronidazole ranges from 20% to 40% in Europe and the USA, with one exception in Northern Italy (14.9%).[100] The overall resistance rate to metronidazole was 33.1% in Europe with no substantial difference between Northern and Southern Europe; however, a noticeably lower prevalence was found in Central and Eastern Europe.[100, 107–111] A higher prevalence has been reported in developing countries (50%–80%), for

example Mexico (76.3%).[110] The prevalence of metronidazole resistance tends to be lower in Japan (9–12%).[94] The prevalence in Canada was reported from 18% to 22%.[112] Metronidazole resistance rates in a US population was recently reported as 21.5%.[85] One possible explanation includes different rates of prior metronidazole use in various countries. As seen in Alaskan Native populations, women have higher rates of metronidazole resistance than men, and prior use of metronidazole was associated with increased metronidazole resistance.[65] The finding of higher rates in Alaskan Native women may be related to antibiotic treatment of gynecological infections, although this was not specifically studied.[65–67]

Other antibiotics

It has been reported that resistance to tetracycline is as low as 0.7% in Spain, 0.5% in the UK and 0.5% in the Hong Kong, or even absent in most countries.[22, 113–115]

The prevalence of resistance to fluoroquinolones has been determined in only a limited number of studies.[1] In China and Italy, levofloxacin resistance rates have been reported as 34.5% and 22.1% respectively.[73, 81] Surprisingly, a recent study from the USA demonstrated a levofloxacin resistance rate of 31.9%; this resistance rate needs to be confirmed with additional studies.[85] Portugal, has reported a high resistance rate of 20.9% in strains isolated from 110 adult patients.[116] Although this resistance to this antibiotic has not been studied as extensively as other antimicrobials, there is a trend of increasing levofloxacin-resistant *H. pylori* (Figure 2c).[22, 67, 72–74, 77, 81–83, 85, 87, 88, 90, 95, 96, 106, 117–121] In the Netherlands, a rate of 4.7% resistance was reported with trovafloxacin, a drug not yet introduced to the Dutch market; this finding suggests cross resistance between the different molecules of this antibiotic group.[122] In France, a rate of 3.3% was reported; in five Eastern European countries, the rate was similar (3.9%).[123, 124] Sitafloxacin appears not to be affected by prior fluoroquinolones use and has also been shown to achieve high *H. pylori* eradication rates.[125, 126]

Resistance to amoxicillin has been shown to be negligible (0 to <2%) in European countries, such as Germany and the Netherlands.[122, 127] In Alaskan Native populations, 6% of patients were infected with amoxicillin-resistant strains of *H. pylori*.[65] In Asia and South America, amoxicillin resistance rates have been reported to be up to 38%.[128, 129] However, it has been suggested that *H. pylori* resistance to amoxicillin does not reduce treatment efficacy.[95]

DIAGNOSIS OF *H. PYLORI* INFECTION

H. pylori testing has become more common over the last decade in patients with epigastric pain and dyspepsia.[130] *H. pylori* is considered a group I carcinogen by the International Agency for Research on Cancer, eradication of which should reduce the incidence of gastric carcinoma and MALT/marginal zone lymphoma.[4, 6, 131, 132] Investigations to assess for *H. pylori* infection are broadly divided into non-invasive and invasive methods.[133–135]

Non-invasive methods for *H. pylori* detection with subsequent treatment is recommended for patients younger than 55 years of age presenting with new-onset dyspepsia without alarm symptoms.[134, 135] These tests include peripheral blood serology, urea breath test and stool antigen test. Serological testing detects immunoglobulin G antibodies to *H. pylori* infection; however, seroconversion for *H. pylori* is rare and serologic tests are not recommended by current guidelines.[1, 136, 137] This test is not suitable for monitoring post-eradication because successful treatment does not alter IgG levels immediately.[138] Additionally, serologic tests may perform differently in different ethnic groups. Serological testing for immunoglobulin M antibodies directed against *H. pylori* can detect active infection, however, IgM levels are only elevated shortly after infection.[139] Urea breath testing is currently the gold standard for determining *H. pylori* status. This test uses either nonradioactive ^{13}C or radioactive ^{14}C to detect urease activity produced by *H. pylori*.[140] One of the advantages of this test is its ability to monitor eradication after treatment. Stool antigen testing detects *H. pylori* antigen in the stool via monoclonal and polyclonal anti-*H. pylori* antibodies.[141] Like the urea breath test, stool antigen testing is capable of monitoring patients in the post-treatment period.[142] This test is a suitable substitute detection modality in areas where the urea breath test is not available.[133, 143]

Upper endoscopy (an invasive procedure) is recommended in the evaluation of all patients presenting with new-onset dyspepsia with alarm symptoms, including unintended weight loss, gastrointestinal bleeding, unexplained iron-deficiency anaemia and progressive dysphagia.[133, 134] Patients with new-onset dyspepsia in the absence of alarm symptoms are recommended to undergo upper endoscopy if they are 55 years of age or greater.[133, 134] However, this age criterion is controversial and may vary by population.[135] Determination of an appropriate age cut-off should take into consideration the presence of persistent symptoms and the local preva-

lence of gastric cancer.[1, 134] If biopsy samples can be easily obtained, the rapid urease test is an option. In this test, two biopsy specimens from the gastric antrum and body are tested to detect the presence of urease from a *H. pylori* infection.[144] Although this test is cheaper than histology, the practice of utilising this test without a specimen also being sent for histopathologic evaluation is not recommended due to the diagnostic yield of histopathology in detecting severity of inflammation and related pathology, including lymphoma or carcinoma.[25] Several ancillary staining methods can be used in conjunction with routine histological evaluation by hematoxylin and eosin stained tissue sections.[145] These include special stains (e.g. modified Giemsa or Warthin–Starry) and *H. pylori* immunohistochemical stains.

ANTIBIOTIC THERAPEUTIC REGIMENS

Multiple therapeutic regimens have been developed in order to treat *H. pylori* infection (Figure 3).[133] If eradication is not achieved, salvage treatment regimens have been proposed.

First line therapy (Table 1)

Triple therapy. The effectiveness of antibiotics is related to many biologic and non-biologic factors including antimicrobial strength, cost, side effects, duration, tolerability of drugs, local antibiotic use, and bacterial resistance.[146, 147] The current standard regimen for *H. pylori* treatment in areas where clarithromycin resistance is below 15% is PPI-based triple therapy which involves the use of a PPI, clarithromycin and amoxicillin.[133, 146, 147] This regimen has been used widely for more than a decade, however, its efficacy has decreased globally.[12] In cases of penicillin allergy, metronidazole can substitute for amoxicillin with equivalent effectiveness to amoxicillin.[146] Increased antibiotic resistance, especially to clarithromycin, is thought to be the main cause of eradication failure for standard PPI-containing triple therapy. Additional factors which may impact cure rates include, but are not limited to, patient compliance, body weight, type of *H. pylori* strains, high bacterial load, gastric acidity and atrophic gastritis.[12, 148] As a result, some authors have suggested avoiding clarithromycin in first-line empiric therapies for areas with high rates of resistance.[59, 149] Per the Maastricht guidelines, sensitivity testing is recommended prior to treatment when *H. pylori* antibiotic resistance rates exceed 20% in the population of interest.[1] In addition, a review of patients' prior antibiotic use is important to increase cure rates as prior exposure to macrolides increase *H. pylori* resistance rates to clarithromycin[65–67]

Nevertheless, 7 days of triple therapy is recommended in areas where the rate of clarithromycin resistance is less than 15% based on European guidelines; 14 days of triple therapy is suggested for areas with clarithromycin resistance >20%.[1, 150] A randomised trial conducted by Paoluzi *et al.* compared *H. pylori* eradication rate between patients treated with 7 days vs. 14 days of standard therapy containing amoxicillin, clarithromycin and omeprazole.[151] Although antibiotic resistance rates were not determined in this study population, the overall results suggested that 14 days of standard therapy resulted in increased eradication (77% vs. 66%).[151]

Bismuth-containing quadruple therapy. Bismuth-containing quadruple therapy consists of a PPI, bismuth and two antibiotics, such as tetracycline, clarithromycin and metronidazole.[1, 133, 152] The regimen has been proven effective as the first line of treatment in areas of high clarithromycin or metronidazole resistance and for patients with recent or repeated exposure to these antibiotics.[1] The main advantage of this regimen is that it can overcome clarithromycin or metronidazole resistance, especially when therapy contains both metronidazole and clarithromycin, concomitantly.[152] A meta-analysis performed by Fisch and Evans has shown better outcomes for bismuth-containing quadruple therapy as compared to the standard triple therapy in areas with increased clarithromycin or metronidazole resistance.[153]

Doxycycline or amoxicillin could be substitutes in countries where tetracycline and bismuth salts are not available, although the efficacy data are conflicting.[153–158] Limited compliance has been reported due to the increased number of drugs and frequent dosing.[159] Quadruple therapy using combination capsules has shown promising eradication rates.[159]

Sequential therapy. Sequential therapy was first introduced by Zullo *et al.* and consists of a 10-day therapy comprising 5 days of PPI plus amoxicillin followed by 5 days of triple treatment of a PPI, clarithromycin and metronidazole.[1, 26, 133] Levofloxacin can be used in patients with penicillin allergy or in areas of high clarithromycin resistance.[160] Although this regimen includes clarithromycin, it is considered an alternative to standard triple therapy in high clarithromycin-resistant areas.[1, 152] This therapeutic regimen employs the use of amoxicillin prior to clarithromycin in order to overcome

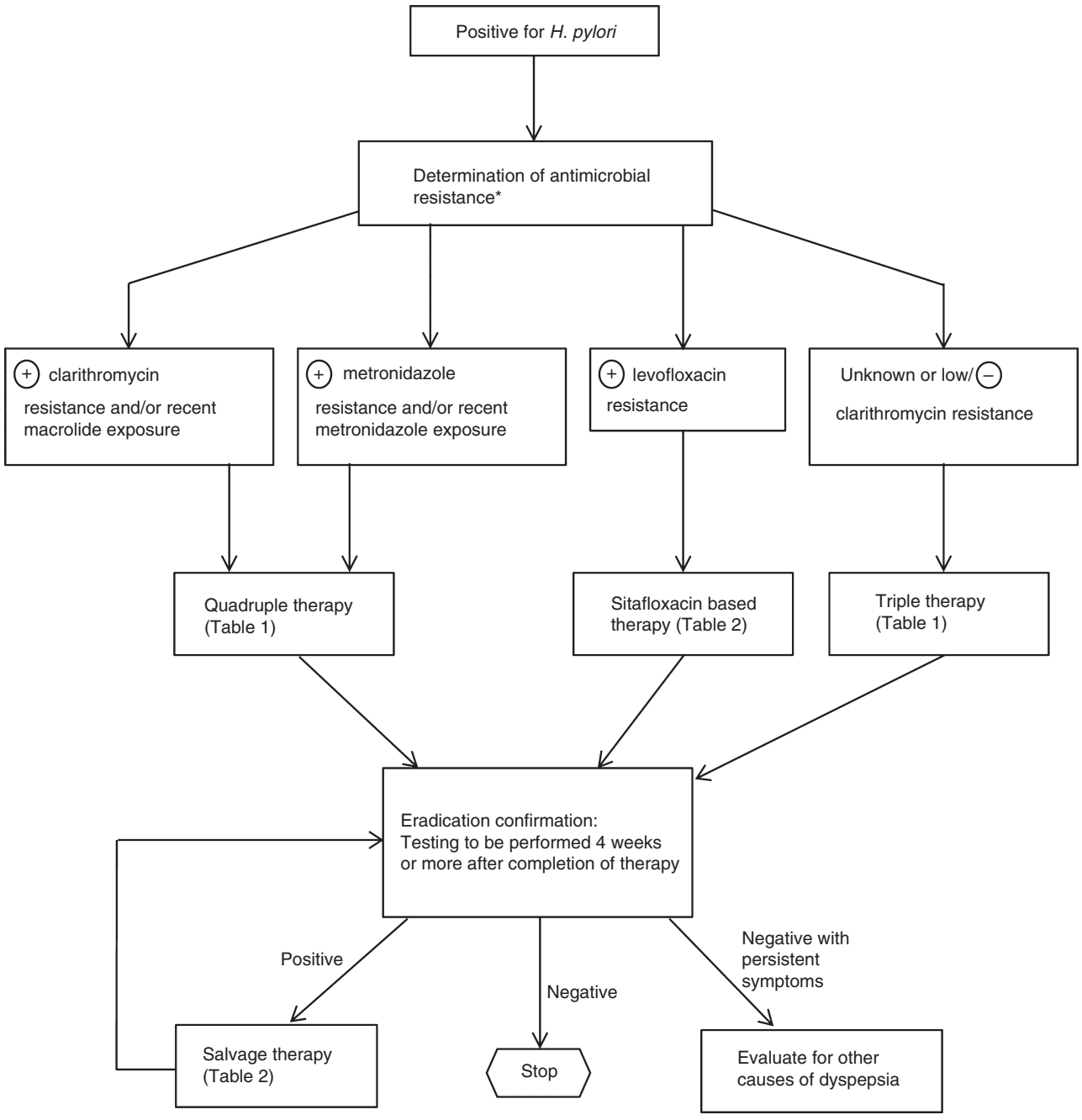

Figure 3 | Recommended algorithm for the management of *H. pylori*.[134]
*In patients who have failed 2 or more courses of therapy, culture and sensitivity testing may be considered.

clarithromycin resistance. Amoxicillin disrupts *H. pylori* cell walls and prevents activation of efflux channels, one of the mechanisms of clarithromycin resistance.[160, 161] The drawback of this therapy is its complex regimen which results in decreased patient compliance.[59, 162] If the patient fails treatment, there is potential to develop multidrug resistance. Additionally, sequential therapy utilises the main antibiotics effective against *H. pylori*, limiting salvage therapy options.

Concomitant therapy. Concomitant therapy consists of a PPI, clarithromycin, amoxicillin and metronidazole for at least 10 days. This regimen has shown superiority over standard triple therapy, especially in cases of

Table 1 | Current initial therapy regimens (Disclaimer: Local resistance rates can markedly impact the choice of therapy)

	Recommendations & comments	Regimen	Duration of therapy
Triple therapy[133]	Consider in areas of low clarithromycin resistance or patients with no prior recent macrolide exposure	• Standard PPI dose b.d. (esomeprazole is q.d.) • Clarithromycin 500 mg b.d. • Amoxicillin 1000 mg b.d.*	10–14 days
Quadruple therapy[133]	Consider in areas of high clarithromycin resistance or patients with recent or repeated macrolide exposure	• Standard PPI dose b.d. (esomeprazole is q.d.) • Bismuth 525 mg q.d.s. • Tetracycline 500 mg q.d.s. • Metronidazole 250 mg q.d.s.	10–14 days
Alternative regimens:			
Sequential therapy[133]	Needs validation in USA	• First 5 days: Standard PPI dose b.d. + Amoxicillin 1000 mg b.d. • Next 5 days: Standard PPI dose b.d. + Clarithromycin 500 mg b.d. + Metronidazole 500 mg b.d.	5 days 5 days
Concomitant therapy[163]	Needs validation in USA	• Standard PPI dose b.d. • Metronidazole 500 mg b.d. • Clarithromycin 500 mg b.d. • Amoxicillin 1000 mg b.d.	10 days
Hybrid therapy[167–169]	Needs validation in USA	• First 7 days: Standard PPI dose b.d. + Amoxicillin 1000 mg b.d. • Next 7 days: Standard PPI dose b.d. + Amoxicillin 1000 mg b.d. + Clarithromycin 500 mg b.d. + Metronidazole 500 mg b.d.	7 days 7 days

PPI, proton pump inhibitor; b.d., twice daily; q.d., daily; q.d.s., four times daily.

* Metronidazole 500 mg b.d. can be substituted for patients with a penicillin allergy.

clarithromycin resistance. A better result with concomitant treatment was observed vs. standard triple therapy in recent randomised clinical trials.[163, 164] A meta-analysis of nine studies (conducted in Japan, UK, Germany, Spain and Italy) showed >90% per protocol analysis (PP) and >80% intention-to-treat (ITT) eradication rates for concomitant therapy, which was an improvement over the standard triple therapy.[162]

Additionally, a recent study conducted in Taiwan evaluated concomitant and sequential therapy regimens using a PPI, levofloxacin, amoxicillin and metronidazole. The population studied was found to have the following resistance rates: levofloxacin – 10.2%, amoxicillin – 0.6%, clarithromycin – 6.6% and metronidazole – 33.5%. ITT analysis showed comparable eradication rates for levofloxacin-based concomitant (92.2%) and sequential (93.3%) therapies.[165]

The advantages of concomitant therapy is the efficacy against dual antibiotic-resistant strains, and the higher compliance rates compared to the sequential therapy.[79, 162, 164, 166] However, it should be noted that the efficacy of concomitant therapy, as with many treatment regimens, depends on the prevalence of *H. pylori* antimicrobial resistance, which varies by geography.

Hybrid therapy (sequential-concomitant therapy). Hsu *et al.* proposed a hybrid therapy which is a combination of sequential therapy and concomitant therapy.[167] The hybrid regimens consist of dual therapy with a PPI and amoxicillin for 7 days, with addition of a concomitant quadruple therapy with a PPI, amoxicillin, clarithromycin and metronidazole for another 7 days. The eradication rate was excellent with 99% in PP and 97% in the ITT analysis, even in dual clarithromycin and

metronidazole resistance strains.[168] A tendency towards better results seems to be the result of extended use of amoxicillin for 14 days, compared to sequential or concomitant therapy. One randomised clinical trial indicated that the hybrid therapy had equivalent eradication rates to 14-day concomitant therapy.[169] However, further validation of these results is needed in order to confirm the effectiveness of hybrid therapy.[169]

Salvage therapy (Table 2)

Quinolone-based therapy. The use of levofloxacin has been proposed as an antibiotic in salvage therapeutic regimens. After failure to eradicate *H. pylori* using standard triple therapy which includes the use of amoxicillin and clarithromycin, levofloxacin-based triple therapy is the most validated regimen used as a second line alternative therapy.[170] This quinolone-based therapy consists of a PPI, levofloxacin and amoxicillin for 10 days. Multiple studies conducted predominantly in Europe and Taiwan reported variable rates of eradication (65–96%), perhaps reflecting varying levels of levofloxacin resistance in the studied populations.[170]

Another quinolone regimen consists of levofloxacin, omeprazole, nitazoxanide and doxycycline (LOAD) for 7 or 10 days. A study consisting of 653 patients conducted by Basu *et al.* found that the LOAD regimen led to a higher rate of eradication when compared to the standard triple therapy, regardless of the duration of LOAD therapy (approximately 90% vs. 73%).[171] Additionally, the study demonstrated that patients following the

Table 2 | Current salvage therapy regimens (Disclaimer: Local resistance rates can markedly impact the choice of therapy)

	Recommendations & Comments	Regimen	Duration of therapy
Quadruple therapy[133]	Consider in patients who were not initially treated with triple therapy	• Standard PPI dose b.d. (esomeprazole is QD) • Bismuth 525 mg q.d.s. • Tetracycline 500 mg q.d.s. • Metronidazole 250 mg q.d.s.	7 days
Levofloxacin triple therapy[133,170]	Needs validation in USA	• Standard PPI dose b.d. (esomeprazole is q.d.) • Levofloxacin 500 mg q.d. • Amoxicillin 500 mg q.d.	10 days
LOAD[171]	Needs further evaluation	• Levofloxacin 250 mg q.a.m. • Omeprazole 40 mg q.a.m. • Nitazoxanide 500 mg b.d. • Doxycycline 100 mg q.p.m.	7–10 days
Rifabutin-based therapy[174]	Adverse effect includes myelotoxicity	• Standard PPI dose b.d. • Rifabutin 150 mg b.d. • Amoxicillin 1000 mg b.d.	10–12 days
Furazolidone quadruple therapy[175]	Needs validation in USA	• Lansoprazole 30 mg b.d. • Tripotassiumdicitratobismuthate 240 mg b.d. • Furazolidone 200 mg b.d. • Tetracycline 1000 mg b.d.	7 days
High-dose dual therapy[55, 177]	Needs validation in USA	• Amoxicillin 750 mg t.d.s. • Lansoprazole 30 mg t.d.s.	14 days
Sitafloxacin therapy[177]	Needs validation	• Sitafloxacin 100 mg b.d. • Metronidazole 250 mg b.d. • Rabeprazole 10 mg b.d.	7 days

PPI, proton pump inhibitor; b.d., twice daily; q.d., daily; q.d.s., four times daily; q.a.m., each morning; q.p.m., each night; t.d.s., three times daily.

LOAD regimen had decreased rates of recurrence at 1-year follow-up.[171]

Levofloxacin-based therapies have also been considered in empiric third-line therapeutic regimens after patients have failed to achieve *H. pylori* eradication with the use of standard first- and second-line therapies. One study proposes the use of levofloxacin in a third-line quadruple therapy in combination with rabeprazole, bismuth subcitrate and amoxicillin. Despite the presence of multi-antibiotic-resistant *H. pylori,* a 10-day course of treatment led to an overall 84% eradication rate. It should be noted that patients who were found to be amoxicillin or levofloxacin-resistant demonstrated lower rates of eradication.[172]

Selection of quinolone therapy should be based on the results of antibiotic susceptibility tests or geographic resistance patterns, as quinolone-resistant strains have increased in concert with the use of quinolones for infections of the respiratory and urogenital tracts. Therefore, this regimen is not generally recommended as first-line treatment.[173] Presently this regimen is used as a second-line treatment in populations where clarithromycin resistance rates are greater than 15%–20% and quinolone resistance rates are less than 10%. It has also been demonstrated that levofloxacin-based triple regimen could be used as an empiric salvage treatment after the failures of first and second-line therapy in low quinolone resistance areas.[1]

Rifabutin-based therapy. Based on *in vitro* studies, rifabutin-based therapy comprising amoxicillin, a PPI, and rifabutin has shown encouraging effects as a salvage treatment for *H. pylori* eradication. However, duration of treatment with this anti-tuberculous agent remains unclear (7-day vs. 10-day vs. 14-day). In addition, rare myelotoxicity is an important complication that needs to be overcome prior to rifabutin's widespread application.[174] Because of the potential for mycobacterial resistance this regimen should be reserved only for rescue treatment. Rifabutin-based triple therapy could be used as an empiric third line salvage treatment and is listed in guidelines for areas in which bismuth and tetracycline are not available, although shortages in the USA have been temporary.[1, 174]

Furazolidone-based therapy. Furazolidone-based therapy is a 1-week therapy with lansoprazole, tripotassiumdicitratobismuthate, tetracycline and furazolidone that has been used as salvage treatment, after second-line treatment failure. This salvage therapy has achieved 90% eradication rate in one analysis.[175] However, the problem with this regimen is the high incidence of side effects and cross resistance with metronidazole.[175]

High-dose dual therapy. High-dose dual therapy has been evaluated in high clarithromycin resistance areas. This regimen consists of a PPI and amoxicillin three times a day for 14 days.[176, 177] In one study patients were randomly given one of two regimens: (i) 1000 mg amoxicillin with 500 mg clarithromycin and 30 mg lansoprazole b.d. for 2 weeks (triple therapy group), (ii) 750 mg amoxicillin with 30 mg lansoprazole t.d.s. for 2 weeks.[176] The eradication rate was 82.8% for standard triple therapy vs. 78.4% for high dose dual therapy, although the difference was not statistically significant.[176] While these results are promising, additional studies to validate and optimise the treatment regimen (including differences between PPI used) are needed before it can be established as a main therapeutic regimen.[178]

ANTIBIOTIC RESISTANCE MECHANISMS

Amoxicillin

Amoxicillin is a moderate-spectrum, bactericidal, beta-lactam antibiotic in the penicillin family. The main mechanisms leading to amoxicillin resistance of *H. pylori* are alterations in penicillin-binding proteins, decreased membrane permeability of antibiotics into the bacterial cell or combinations of these resistance strategies. Expression of active efflux pumps that excrete drugs and point mutations in the *pbp1A* gene may contribute to the mechanisms of resistance to beta-lactams. Other mutations such as *pbp2, hefC, hopC and hofH* have been identified among *H. Pylori*-resistant strains.[179]

Clarithromycin

Clarithromycin is a bacteriostatic antibiotic that inhibits bacterial protein synthesis by reversibly binding to the 50S ribosomal subunits. The 50S ribosomal subunit is itself composed of 23S ribosomal RNA, 5S ribosomal RNA, and RNA binding proteins. The peptidyl transferase loop of the V domain of 23S ribosomal RNA molecule is the target site of clarithromycin. Resistance to clarithromycin is generally caused by point mutations in the 23S rRNA gene, the most frequent is A2143G (69.8%), followed by A2142G (11.7%) and A2142C (2.6%).[79] These mutations prevent the macrolide from binding. Moreover, other mutations such as A2115G, G2141A, C2147G, T2190C, C2195T, A2223G and C2694A have been implicated in the development of

clarithromycin resistance, although their precise role in the mechanism of resistance remains unclear.[180]

Metronidazole

Metronidazole, a bactericidal antibiotic, is a synthetic nitroimidazole. This prodrug is activated by nitroreductases within the cytosol of the microorganism, producing a toxic metabolite. In metronidazole-resistant strains, null mutations in the *rdxA* gene have been identified. The genecodes for an oxygen-insensitive NADPH nitroreductase (RdxA), whose expression is necessary for intracellular activation of the drug, and the mutations causes inactivation of these nitroreductases. Nonetheless, a number of resistant strains have been reported, such as *frxA* (coding for NADPH flavinoxidoreductase), and *fdxB* (coding for ferredoxin-like enzyme), which can also confer resistance to metronidazole.[181–183]

Tetracycline

Tetracycline is a bactericidal antibiotic which inhibits protein synthesis by binding to the 30S subunit of ribosomes; this blocks the binding of aminoacyl-tRNA, resulting in stalled synthesis of nascent peptide chains. Antibiotic resistance to tetracycline is due to an enhanced energy-dependent efflux of tetracycline-cation complexes across the cell membrane by membrane-associated efflux proteins which decreases the intracellular concentration of tetracycline.[184] Conversely, sensitivity to tetracycline is increased by deletions in these efflux genes.[184]

Another mechanism of tetracycline resistance is mediated through ribosomal protection proteins. These protection proteins increase tetracycline resistance either by decreasing the affinity of ribosomes for tetracycline or by releasing the bound antibiotic from the ribosome. Additionally, two other mechanisms have been reported including enzymatic inactivation of tetracycline and point mutations in the 16S rRNA genes that affect the binding site of tetracycline.[184]

Levofloxacin

Point mutations of *gyrA*, which codes for DNA gyrase, have been identified in the quinolone-resistant determination region with the major mutations being found at position in the codons coding for amino acid 87, 88, 91 or 97, conferring resistance of levofloxacin and other quinolones.[185, 186] However, studies have shown that sitafloxacin may overcome the resistance conferred by these point mutations.[187, 188]

Rifabutin

Resistance to rifabutin is generally caused by point mutation in codons 524–545 or codon 585 of the *rpoB* gene.[189, 190] Moreover, cross resistance between rifabutin and rifampin has been reported.[191]

TECHNIQUES FOR DETECTING *H. PYLORI* ANTIBIOTIC RESISTANCE (TABLE 3)[184–186, 189, 192, 193]

Endoscopic-guided antibiotic susceptibility testing has been suggested not only for treatment after failure of second-line therapies but also for determining antibiotic susceptibility prior to the administration of a first-line clarithromycin-containing therapy, especially in areas of high clarithromycin resistance.[194–196] Currently, several techniques exist for evaluating antibiotic resistance in *H. pylori*, but differ in the timing and character of the requisite analytic specimen.

Culture-based techniques

In vitro susceptibility testing of *H. pylori* using agar dilution method are practical for testing large numbers of strains; it is not suitable for the testing of small numbers of strains on an ongoing basis.[194–197] The Epsilometer test (*E*-test) method involves the use of test strips applied to an inoculated agar plate in order to determine the antibiotic's minimum inhibitory concentration.[198] One study found the *E*-test produced reproducible results in determining the sensitivity of *H. pylori* isolates to ampicillin, clarithromycin and metronidazole.[198, 199] From an international perspective, *E*-test appears to be a suitable method for determining *H. pylori* antibiotic sensitivity.[199–201] However, the availability of the *E*-test strips for one of the key antibiotics of interest, clarithromycin, is currently not globally available for clinical use.

One of the main drawbacks of both the agar dilution and *E*-test is that they only test a single *H. pylori* strain. In areas of high *H. pylori* prevalence and increased likelihood of patients being infected with multiple *H. pylori* strains, these two testing modalities may fail to provide complete antimicrobial resistance data.

Molecular techniques

The gold standard methods of antibiotic resistance are based on phenotypic methods performed by the agar dilution method.[194, 202] These methods, however, can take up to 2 weeks to be completed. Detection of point mutations using molecular methods was developed in part to shorten the turn-around time. In addition,

Table 3 | Current techniques for detecting *H. pylori* antibiotic resistance

Name of the method	Basis for method	Sensitivity	Specificity	Advantages of the method	Disadvantages of the method
Agar Dilution Method	Based on phenotypic methods failure of second-line therapies	–	–	Adaptable for the testing of large numbers of strains	Technically demanding Time consuming
Epsilometer Test (E- test) Method[184]	Based on phenotypic methods	45%	98%	Adaptable for testing of small numbers of strains Less technically demanding	Time consuming
PCR-based methods[185]	Based on detection of point mutations	98%	92%	High-sensitivity Rapid detection of microorganisms	Affected by DNA contamination
FISH-Based Method[186]	Fluorescent-labelled DNA probes to identify DNA sequences on chromosomes	97%	94%	Time-saving Accurate Cost-effective	Degradation of the probe by proteases Inability to penetrate the bacterial cell wall (fresh tissue)
PNA-FISH-Based Method[189]	Fluorescently-labelled PNA probes to identify DNA sequences on chromosomes	80%	93.8%	Ability to penetrate the bacterial cell wall Resistant to nucleases and proteases	Not widely available for standardisation
Line Probe Test[192,193]	DNA-based test to identify multiple variants simultaneously. Commercialised as kits for laboratories.	100%	86.2%	Fast, standardised test that examines both clarithromycin and fluoroquinolones resistance	Not available in the USA

molecular techniques can often use either fresh or formalin-fixed samples.

Real-time PCR has been used to successfully determine *H. pylori* susceptibility to clarithromycin.[203, 204] Additionally, PCR using formalin-fixed paraffin-embedded samples has been shown to reliably detect the *H. pylori* 23S rRNA mutations associated with clarithromycin resistance.[86] Another advantage of PCR is the potential to gather complete antimicrobial resistance data in patients infected with multiple strains of *H. pylori*. Although the use of PCR-based methods provides rapid detection of micro-organisms, these techniques can be affected by DNA contamination or degradation since the high sensitivity of these methods often result in the detection of dead or nonculturable microorganisms.[205]

Fluorescence in situ hybridisation (FISH) is a time-saving, accurate and cost-effective method for the detection of antibiotic resistance in cultured *H. pylori* colonies. This method can be used directly on biopsy specimens procured for histopathological and microbiological examination, allowing for rapid detection of *H. pylori* resistance without requiring DNA preparation.[201, 206] The results can theoretically be available within 3 hours after an endoscopy by utilising frozen tissue sections.[205] The limitations of this method include the degradation of the probe by proteases and nucleases present in the sample and poor accessibility of the microbial cell wall for the probes.

Recently, peptide nucleic acid (PNA) probes using FISH have been used for the detection of several bacteria in lieu of the typical DNA molecular probes.[175, 207] PNA molecules are DNA mimics with high affinity for DNA or RNA complementary sequences.[208, 209] PNA probes are normally relatively small (13–18 nucleotides), increasing their ability to penetrate the bacterial cell wall. Moreover, the PNA molecules are more resistant to nucleases and proteases than DNA molecules.

CONCLUSIONS AND FUTURE DIRECTIONS

Helicobacter pylori infection remains a very common worldwide condition with strong geographic variations and the prevalence of antibiotic resistance appears to be rapidly increasing. This is particularly evident in countries such as Japan, Korea and China, in which antibiotic resistant strains of *H. pylori* have been studied most extensively (Figure 2). Indeed, many countries have crossed the 15–20% threshold for antibiotic resistance over the past 20 years (Figure 2). During this period of increasing antibiotic resistance, the eradication rate of empiric therapy for *H. pylori* has dropped below the

80–90% target, and failure rates range from 29% in the USA to 40% in Western Europe and Japan.[25, 210, 211] Treatment failure is multifactorial and requires further study, but is likely due to poor compliance and antibiotic resistance. In addition, reinfection rates are unclear and may influence measured treatment failures.[212, 213] Variable antibiotic susceptibility of different *H. pylori* strains found within the antrum of the stomach vs. the corpus may also be contributing to treatment failure.[25, 127] The cagA status of bacterial strains is also a risk factor for treatment failure as *H. pylori* eradication was more successful in patients harbouring cagA+ strains compared with those infected with cagA− strains.[212, 214] This finding may explain why cure rates are higher in patients with peptic ulcer disease than those with non-ulcer disease.[212, 215] Additionally, patients who fail first-line therapy have been shown to develop secondary resistance to the recommended antibiotics, further promoting the selection of antibiotic-resistant *H. pylori* strains.[216]

Theoretically, the widespread general usage of antibiotics by the public may be further contributing to the development and increased prevalence of antibiotic-resistant strains of *H. pylori*. Globally, antibiotic consumption increased by 36% between 2000 and 2010, mainly in developing countries. Perhaps even more importantly, one of the largest absolute increases in consumption was observed for broad-spectrum antibiotics. This overall increase in antibiotic consumption also includes a global increase in macrolide consumption by approximately 20% over this time period.[217] As antibiotic resistance has been correlated with antibiotic usage, the continual increase in usage of broad-spectrum antibiotics in recent years suggests that rates of *H. pylori* antibiotic resistance might be increasing in parallel.[59, 150] Importantly, the prior usage of macrolides and, specifically, clarithromycin has been shown to directly impact the development of antibiotic-resistant strains of *H. pylori*.[22, 64, 65]

Because of the clinical importance of decreased eradication rates, regional variation in antibiotic resistance rates and the marked increase over time of antibiotic resistance rates in the past 15 years, there is a critical need to determine the current rates of local antibiotic resistance. Such a determination would not only facilitate the selection of appropriate antibiotic treatment regimens but also serve as a potential basis for transitioning to individualised analysis of antibiotic resistance prior to definitive treatment. Therefore, a paradigm shift in therapy towards patient-specific tailoring of effective antibiotic treatment strategies may lead to reduced treatment failures and stem the tide of increasing *H. pylori* antibiotic resistance in various populations throughout the world.

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All authors approved the final version of the manuscript.

ACKNOWLEDGEMENTS

We would like to thank Dr. John W. Thomas for his suggestions and proofreading of this article.

Declaration of personal interests: (i) JP has served as an advisory board member for Fujirebio and Targeted Diagnostics and Therapeutics. MV has served as a consultant for AbSci and Celgene. SC has served as an author for UpToDate. No research funding has been received related to this study. (ii) JP is an employee of UT Southwestern Medical Center. HA, IT, VV, MV, SG, and SC are employees of UC San Diego Health System. (iii) None related to this study. (iv) None related to this study.

Declaration of funding interests: None.

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