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TOPIC HIGHLIGHT

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# Treatment of *Helicobacter pylori* infection: Meeting the challenge of antimicrobial resistance

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## Abstract

Treatment of *Helicobacter pylori* (*H. pylori*) infection is paramount for the management of prevalent gastrointestinal disorders including peptic ulcer disease and gastric cancer. Due to the wide increase in prevalence of *H. pylori* resistance to antibiotics, clarithromycin-based triple therapies are not any more suitable for unconditional empiric use, and should not be recommended, unless local resistance to this antibiotic is low (< 20%). Alternative strategies have been proposed to overcome the issue of increasing clarithromycin resistance, and some of them are already implemented in clinical practice. These comprise: (1) adoption of novel, more effective, empirical treatments: bismuth quadruple, sequential, non-bismuth quadruple (concomitant), dual-concomitant (hybrid), and levofloxacin-based regimens, the latter mainly designated as second-line/rescue options; (2) perspectives for a susceptibility-guided (tailored) therapeutic approach based on culture-free molecular testing methods; and (3) adjunct use of probiotics to improve eradication rates. The present article is aimed to provide a comprehensive overview of current and emerging strategies in the treatment of *H. pylori* infection, focusing on the challenge of antimicrobial resistance.



**Key words:** *Helicobacter pylori*; Antibiotic resistance; Bismuth-quadruple; Concomitant; Sequential

**Core tip:** Rising clarithromycin resistance has accounted for a dramatic decline in the efficacy of standard therapies for *Helicobacter pylori* (*H. pylori*) infection. Bismuth-quadruple, sequential, non-bismuth quadruple (concomitant), dual-concomitant (hybrid), and levofloxacin-based regimens are now recommended as preferred empirical treatments (> 90% efficacy). However, empiric treatment of *H. pylori* is likely to become more challenging as even these improved regimens are prone to the effect of antibiotic resistance. Individualized therapy appears as a reasonable future alternative, currently limited by the shortcomings of performing culture. Advances in the genotypic characterization of *H. pylori* therapeutic susceptibility are likely to revolutionize our approach to tailored treatment.

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## INTRODUCTION

*Helicobacter pylori* (*H. pylori*) is an ubiquitous human patho-

gen, infecting approximately one half of the world's population and up to 80% in developing countries[1]. After colonizing the gastric mucosa, usually during childhood, *H. pylori* play a causal role in the development of chronic gastritis and peptic ulcer disease[2,3]. Moreover, *H. pylori* is a well-recognized carcinogen, primarily involved in the development of gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma (MALT)[4]. In parallel with accumulating evidence on its pathogenicity, the ability to reliably eradicate *H. pylori* has been established as a major step in the management of prevalent gastrointestinal disorders, including peptic ulcer disease, functional dyspepsia and low-grade MALT[2,3,5,6]. Nonetheless, eliminating the infection represents the most consistent strategy to prevent gastric cancer[3,7]. Apart from gastrointestinal disorders, extra-digestive conditions are now included as indications to treat *H. pylori*: idiopathic thrombocytopenic purpura, vitamin B12 deficiency and unexplained iron deficiency anemia[8]. Additional associations are emerging, including colorectal disease and cancer, ischemic heart disease and neurological disorders, although no clear therapeutic link is as yet available for these conditions[9,10].

Culturing the pathogen is a common step in the treatment of bacterial infections, but this has not been the case for *H. pylori*, for which treatments have been routinely prescribed empirically. This is due to the fact that performing endoscopy and *H. pylori* culture is neither widely available, nor well-tolerated by all patients, and furthermore it is time-consuming and costly[11]. For the last 2 decades, standard triple therapies comprising of a proton pump inhibitor (PPI) *bid*, amoxicillin 1000 mg *bid* and clarithromycin (CAM) 500 mg *bid* or metronidazole (MNZ) 500 mg *bid*, all given for 7-14 d, represented the standard of care regimens to empirically eradicate *H. pylori*. The high eradication rates (> 90%) provided by these treatments during the 90's, together with their relative simplicity and optimal safety profile, have accounted for their enthusiastic acceptance in expert panels and consensus recommendations worldwide[12-15]. However, in following years, the efficacy of legacy triple regimens has been seriously challenged and eradication rates lower than 70% are now reported in many countries[16-19]. These elusive success rates preclude acceptability under Maastricht consensus [80% in intention to treat (ITT) analysis] and fall short of what it should be expected for an infectious disease, for which a 95% per protocol (PP) efficacy is warranted[20]. This will avoid exposing the patient in repeated treatment courses resulting in both multiple side effects (and therefore poor patient adherence and quality of life) and spreading of secondary antibiotic resistance. Although a series of both host- and pathogen-related factors may affect performance of legacy treatments[21-23], a worldwide increase in the levels of *H. pylori* resistance to antibiotics, especially that to CAM, is the most important determinant of failure of standard triple therapies[24].

The present review provides a comprehensive overview of *H. pylori* eradication focusing on current and emerging approaches to the issue of increasing antimicrobial resistance.

## *H. PYLORI* RESISTANCE TO ANTIBIOTICS

A class-wide resistance to macrolides is the result of point mutations in three adjacent nucleotide positions (*A2143G*, *A2142G* and *A2142C*) in the peptidyl transferase loop of the *23S*rRNA gene[25,26]. Although these three point mutations account for 90% of cases of primary CAM resistance in Western countries, each of them is individually associated with different minimal inhibitory concentration (MIC) values for CAM resistance (assessed by *H. pylori* culture *in vitro*), suggesting a different impact on the determination of phenotypic CAM resistance[27]. Indeed, a lowest eradication rate (30.7%) has been observed when the phenotypic bacterial resistance was genetically linked to *A2143G*, suggesting this mutation, rather than the *A2142G* and *A2142C*, may significantly affect the therapeutic outcome[28]. In addition to 23SrRNA point mutations, an active multidrug efflux mechanism, responsible for rapidly transferring the drug out of the bacterial cell, is associated with the development of CAM resistance[29].

In a recent systematic review, the global incidence of primary *H. pylori* resistance to CAM has been reported to be as high as 17.2%, showing an increasing trend worldwide[30]. An overview of the continental (America, Europe and Asia) distribution of *H. pylori* antibiotic resistance is shown in Figure 1. Epidemiology of *H. pylori* susceptibility remains scarcely documented in Africa, with some studies suggesting extremely high rates[31] in contrast to rates as low as 1% of CAM resistance recorded by other[32]. Indiscriminate consumption of macrolides is likely the main reason for the consistent increase in CAM resistance rates[33,34]. Congruently, a positive anamnesis of respiratory tract infections was identified as an independent predictor of CAM resistance in a Bulgarian study[34], and different antibiotic consumption policies may, at least partially, explain geographical variations in *H. pylori* antimicrobial resistance. For instance, a 49% of CAM resistance has been reported in some Spanish areas, but only 1% in the Netherlands, reflecting a stricter Northern European policy for antibiotic use[33]. Such significant variation in the CAM resistance rates between Northern (< 10%) and Southern, or Western/Central European countries (> 20%), has been recently confirmed in a prospective assessment of *H. pylori* antimicrobial resistance including 18 European countries (2008-2009)[33]. In this same study (2204 patients), CAM-resistance was determined to 17.5% and was significantly associated with the use of long-acting macrolides only. Similarly, prevalence of CAM resistance has increased for 12.8% to 23.8% between 2000 and 2009 in China, whereas a consistent increase from 7% to 15.2% has been recorded in Japan[35,36]. Contrarily, prevalence of CAM resistance is still low (< 10%) in some developing countries (*e.g.*, Bangladesh, Malaysia), and elsewhere (*e.g.*, Sweden, Taiwan, Croatia),

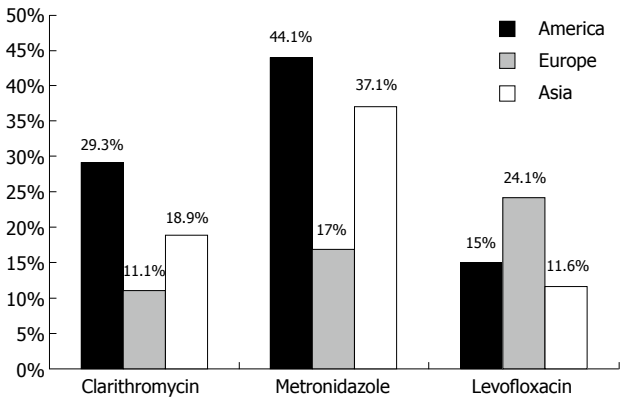


**Figure 1 Continental rates of *Helicobacter pylori* resistance to clarithromycin, metronidazole and levofloxacin.**

probably due to the low macrolide consumption or later introduction of newer macrolides in these areas[37]. However, as antibacterial resistance is a strictly local phenomenon, a wide geographic variation has been observed and a patchy distribution is possible even within the confines of a single country. For instance, resistance to CAM was found to vary from 0% to 25% among Italian regions[38]. This wide variation is clearly challenging efforts for standardization of anti-*H. pylori* regimens. Additional factors such as phylogeographic features of *H. pylori* strains may contribute to the significant geographical variation in prevalence of antibiotic resistance as well as differences in the virulence of *H. pylori* infection[39].

The deleterious impact of CAM resistance on the efficacy of standard treatments has been well-documented in clinical trials. In the most consistent pooled data analysis (20 studies, 1975 patients), standard treatments were only 18% effective against CAM-resistant strains[40]. In a meta-analysis by Fischbach and Evans, the success of triple therapy was decreased by 66.2% (95%CI: 58.2%-74.2%) when strains of *H. pylori* were resistant *vs* susceptible to CAM[41]. A more recent analysis revealed similar results: including antimicrobial susceptibility data from 4 randomized clinical trials (RCTs)[18,42-44], standard triple therapies successfully eradicated 88% and 14% of CAM-sensitive and CAM-resistant *H. pylori* strains respectively (risk difference = 0.75, 95%CI: 0.63-0.87)[45].

MNZ is another agent frequently included in regimens to eradicate *H. pylori* and, if so, presence of MNZ-resistance may also affect the therapeutic outcome. Mechanisms of MNZ resistance are complex and are largely associated with inactivating mutations of the *rdxA* and *frxA* genes encoding reductases which are required for the activation of MNZ[46]. However, development of MNZ resistance is known to be possible independently to these mutations, suggesting the involvement of alternative resistance mechanisms[47]. Recently, prevalence of *H. pylori* resistance to MNZ has been estimated to 17%-44% for Europe and America, it is highest in Africa (up to 80%-92.4%, probably due to the wide use of MNZ for parasitic infections), whereas lower rates have been reported in Japan (9%-12%)[30,40]. Contrarily, in China, an explosive increase in MNZ resistance from 23.8% to 56.6% has been recorded in the last decade[35]. It has been postulated that resistance to MNZ accounts for a drop in efficacy of up to 50% for either bismuth- and PPI-containing triple therapies[48]. However, in contrast to CAM-resistance, resistance to MNZ can be largely overcome by increasing dose and prolonging duration of therapy, thus it is generally considered less important clinically[49]. Critically, evaluation of MNZ resistance by relying on Etest® (AB bioMerieux; Solna, Sweden) is inaccurate leading to overestimation of the true levels[50]. Confirmation by using the more laborious agar dilution method is therefore required to avoid misclassifications.

Less data is available with respect to quinolone resistance. As for CAM, development of levofloxacin resistance reflects use of the drug, frequently for the treatment of urinary tract infections[34]. It involves point mutations in the quinolone resistance-determining region in the gyrase A (*gyrA*) gene preventing binding between the antibiotic and the enzyme[51]. Recently, prevalence of levofloxacin resistance has been reported to exceed 20% in Europe and even 15% in America (Figure 1). On the contrary, levofloxacin resistance seems to be lower in Asia (11.6%), despite there is a substantial variation from a highest of 14.9% in Japan to a lowest of 2.6% in Hong Kong[30]. Age may be a useful predictor of resistance presence, as levofloxacin resistance is less frequently encountered in subjects < 45 years old[52]. Interestingly, Dutch investigators reported a trovafloxacin resistance rate of 4.7%, in spite of the fact that this agent was not yet introduced in the Netherlands[53]. Such finding is suggestive of cross-resistance between different quinolone agents[51,54].

Fortunately, resistance to amoxicillin is exceptional and generally do not constitute a relevant clinical problem.

## OVERCOMING *H. PYLORI* RESISTANCE TO ANTIBIOTICS

As outlined by the recent European Maastricht IV/Florence consensus report, standard triple therapies are not any more recommended for unconditional empiric use[8]. Instead, use of standard regimens should be adapted to the local resistance pattern (*i.e.*, used only if local CAM resistance is < 20%), or rely on susceptibility testing provided that pre-treatment culture is available (*i.e.*, used as tailored treatments). Alternative strategies have been proposed, and some of them are already implemented in clinical practice, aiming to overcome the problem of antimicrobial resistance. These comprise: (1) development of novel, more effective, empirical treatments; (2) perspectives for a tailored therapeutic approach based on pre-treatment determination of *H. pylori* therapeutic susceptibility; and (3) adjunct use of probiotics to improve eradication regimens. Each of these developments and advances in the field of *H. pylori* infection therapies will

**Table 1 Currently recommended regimens for the treatment of *Helicobacter pylori* infection**

| Treatment | Regimen | Comment |
|---|---|---|
| First-line therapies | | |
| Standard triple therapy | A PPI (standard dose, *bid*), amoxicillin (1 g, *bid*) and clarithromycin (500 mg, *bid*) for 14 d | Widely used option<br>Only suitable for areas with < 20% incidence of cam resistance or as tailored treatment. |
| Bismuth-containing quadruple therapy | A PPI (standard dose, *bid*), bismuth (standard dose, *qid*) tetracycline (500 mg, *qid*) and metronidazole (500 mg, *qid*) for 10-14 d | Works independently to CAM and largely overcome MNZ resistance<br>Valuable second-line treatment after failure of CAM-based regimens<br>Patient-friendly monocapsule available<br>Suitable for patients with penicillin allergy<br>Non-availability of bismuth and/or tetracycline in some countries |
| Sequential therapy | A 5-d dual therapy with a PPI (standard dose, *bid*) and amoxicillin (1 g, *bid*) followed by a 5-d triple therapy with a PPI (standard dose, *bid*), clarithromycin (500 mg, *bid*) and metronidazole (500 mg, *bid*) | Widely evaluated option<br>Probably effective in high resistance settings<br>Questionable efficacy against double-resistant strains<br>Less satisfactory results in more recent studies contacted outside Italy |
| Non-bismuth quadruple "Concomitant" therapy | A PPI (standard dose, *bid*), clarithromycin (500 mg, *bid*), amoxicillin (1 g, *bid*) and metronidazole (500 mg, *bid*) for 10 d | Probably effective in high resistance settings<br>Larger number of pills compared to sequential and hybrid therapies |
| Hybrid therapy | A 7-d dual therapy with a PPI (standard dose, *bid*) and amoxicillin (1 g, *bid*) followed by a 7-d quadruple therapy with a PPI (standard dose, *bid*), amoxicillin (1 g, *bid*), clarithromycin (500 mg, *bid*) and metronidazole (500 mg, *bid*) | Probably effective in high resistance settings<br>Few data available on its efficacy/safety |
| Second-line/rescue therapies | | |
| Levofloxacin-based triple therapy | A PPI (standard dose, *bid*), levofloxacin (500 mg, *bid*) and amoxicillin (1 g, *bid*) for 10 d | Works independently to CAM and MNZ<br>Ineffective for high quinolone resistance settings (> 10%)<br>Rapid development of quinolone resistance |
| Rifabutin-based triple therapy | A PPI (standard dose, *bid*), rifabutin (150 mg *bid*) and amoxicillin (1 g *bid*) for 14 d | Third or more rescue option<br>Significant safety issues<br>Development of mycobacterium resistance |

PPI: Proton pump inhibitor; CAM: Clarithromycin; MNZ: Metronidazole.

be discussed below.

## EMPIRICAL TREATMENTS EXPERIMENTED TO OVERCOME CAM RESISTANCE

Although based on the same key antibiotics (CAM, MNZ and levofloxacin), these improved regimens are largely validated (PP eradication > 90%) in settings of high CAM resistance and are now recommended as first-line empirical therapies. An overview of currently recommended regimens to eradicate *H. pylori* is shown in Table 1.

Bismuth quadruple therapy (BQT) works independently to CAM and levofloxacin, *i.e.*, the two problematic, in terms of resistance development, compounds. It is not completely novel but rather represents an enhanced evolution of the old regimen comprising a bismuth salt, tetracycline and MNZ[55], in which addition of a PPI, increase MNZ dose (1500-1600 mg/d) and prolonged treatment duration (10-14 d) are successfully limiting the impact of MNZ resistance. BQT is now designated as a preferred first-line empirical treatment achieving > 90% eradication in presence of CAM resistance and > 85% in regions with a high MNZ resistance[56]. Increased efficacy against MNZ-resistant strains, which offsets the ability of standard therapies to overcome CAM resistance, is likely the key for the improved performance with BQT[57]. To solve the issue of taking a large number of pills, a galenic three-in-one formulation (containing bismuth, MNZ and tetracycline in a single capsule) has been proposed (Pylera®; Aptalis, Mont St Hilaire, QC, Canada)[58]. Efficacy of the monocapsule formulation was addressed in two large randomized control trials (RCTs), conducted in North America[43] and Europe[18], showing ITT eradication rates of 86% and 80% respectively. Contrarily, the superiority of BQT over standard triple therapy was questioned in a recent meta-analysis, in which both treatments yielded suboptimal results (ITT eradication: 77.8% for BQT and 77% for standard therapy), although there was a significant heterogeneity among studies, especially concerning MNZ dosing[57]. BQT is also a valid, and cost-effective, rescue option after failure of CAM-based regimens. A second-line, high MNZ-dose (2 g/d) BQT regimen yielded a 90.8% PP efficacy in a Taiwanese study[59], and 93.1% was obtained in yet another Asian study using standard MNZ dose (1600 mg/d) conducted in a high MNZ-resistance setting (96.8%)[60]. In Greece, a high MZN-resistance country, 84% of second-line efficacy was recorded by using BQT[61]. In a meta-analysis, the average ITT eradi-

cation using BQT after failure of standard triple therapy was 77%, even though in 19 out of the 30 studies duration of treatment was inherently short (7 d)[62]. Notably, retrying BQT after its own failure may be worthy[63]. The potential toxicity of bismuth as well as non-availability of bismuth salts or tetracycline in some countries are the main shortcomings related to BQT. Attempts to substitute tetracycline by either amoxicillin or doxycycline yielded elusive results[64,65]. Of note, a recent meta-analysis (4763 patients) questioned a suboptimal safety of bismuth showing a comparable tolerability between bismuth-containing *vs* non-bismuth regimens, except from dark stools being more common in the bismuth group[66]. In a recent multicenter study from Spain, BQT was an acceptable third line option (65% in ITT and 67% in PP analysis) after two previous eradication failures with CAM- and levofloxacin-containing triple therapies. Adverse events were reported in 22% of patients, nausea (12%), abdominal pain (11%) and metallic taste (8.5%), but none of them severe[67].

Proposed by Italian investigators, sequential therapy (ST) is another novelty recommended as first-line therapy for high CAM-resistance settings[68]. It involves the same key antibiotics used in standard treatments, but given sequentially. By disrupting the bacterial wall, initial administration of amoxicillin has been suggested to prevent the development of efflux channels which rapidly transfer CAM out of the bacteria[69]. Several RCTs and meta-analyses reported on the superiority of ST over standard treatments in settings of high resistance to either CAM or MNZ[70-73]. Including 15 RCTs (3346 patients), ST displayed an overall eradication rate of 91.7% (95%CI: 90%-93%, ITT analysis) *vs* 76.7% (95%CI: 75%-79%) yielded by standard therapy. By pooling data from 4 studies, the efficacy of ST against CAM-resistant *H. pylori* strains ($n$ = 55) was 75%[73]. However, later studies conducted outside Italy (where most of the initial trials were coming from) provided discouraging results: in a large South-American RCT[74], the probability of successful eradication was 5.6% (95%CI: -0.04%-11.6%) higher using a 14-d ST (82.2%) *vs* 10-d ST (76.5%). Similarly, eradication rates < 80% were shown in studies conducted in Iran and South Korea[75,76], contrarily to a RCT from Taiwan (90.7% and 87% of eradication success with a 14- and 10-d ST)[77], which however is known to be a country of low (< 20%) CAM resistance. In a very recent Asian meta-analysis (17 RCTs, 3419 participants), a 10-d ST appeared superior to legacy treatment [81.8% (95%CI: 78.9%-84.6%) *vs* 74.3% (95%CI: 69.6%-78.8%)], although the pooled efficacy was lower than results from earlier European studies[78]. Despite ST seems fairly effective against mono-resistant strains, a decreased efficacy against double-resistant (CAM and imidazole) *H. pylori* strains may compromise use of ST in high-resistance areas[79]. Including 8 studies with antibiotic susceptibility data, Gatta *et al*[80] analyzed the effect of antimicrobial resistance on the eradication rates provided by ST. They found that ST was able to eradicate 72.8% (range: 61.6%-82.8%) of CAM-resistant strains and 86.4% (range 78%-93%) of MNZ-resistant strains, but only 37% (range 16.2%-60.7%) of dual-resistant *H. pylori* strains. However, as stated by the same authors, these results should be interpreted with caution due to low number of patients with antimicrobial susceptibility (91/192/34 with CAM/MNZ/double-resistant strains treated with ST). Crucially, in this consistent meta-analysis (including 46 RCTs), a 10-d ST (overall eradication rate 84.3%, 95%CI: 82.1%-86.4%) was proven superior to 7-d triple therapy (RR = 1.21, 95%CI: 1.17-1.25), marginally superior to 10-d triple therapy (RR = 1.11, 95%CI: 1.04-1.19), but not superior to either a 14-d triple therapy (RR = 1, 95%CI: 0.94-1.06) or bismuth-based therapy (RR = 0.99, 95%CI: 0.94-1.05).

A non-bismuth quadruple therapy (NBQT), also called "concomitant" therapy, of short (3-5 d) duration was originally proposed in 1988 by German and Japanese investigators[81,82]. It returns nowadays as an effective first-line option in areas harboring high CAM resistance, but with prolonged treatment duration (10-d), as this seems a reasonable strategy to maximize cure rates at no cost in terms of safety[83]. Data on its efficacy (> or close to 90%) and safety, as well as superiority over legacy triple therapy, has been provided in several trials[84-88] and proven on a meta-analytic base. In a 2012 meta-analysis by Gisbert *et al*[83] (19 studies, 2070 patients), the overall cure rate provided by NBQT was 88% (95%CI: 85%-91%), increased to 91% when 3 outlier studies were excluded. Worthy of note, in many of the included trials duration of treatment was 3-5 d. However, as in a previous meta-analysis[89], the authors noted a trend toward better results with longer (7 or more days) treatments. Evaluation of 5 RCTs comparing NBQT *vs* standard therapy revealed superiority of the quadruple regimen with a pooled eradication difference of about 11%[89]. Crucially, a combination of extra-prolonging treatment duration (14 d) and use of a high PPI dose (omeprazole 40 mg × 2) may significantly boost cure rates as demonstrated in a non-inferiority Spanish multi-center trial were > 95% (PP) efficacy was obtained[90]. Comparison between ST and NBQT is paramount, as both treatments are relevant competitors. In a recent back to back comparison (338 patients from 11 hospitals) performed in a country with high CAM resistance (Spain), NBQT showed a non-significant advantage over ST (OR = 1.5, 95%CI: 0.9-2.8)[91]. Crucially, although both regimens seem reasonably effective against mono-resistant strains, NBQT has been suggested to be more effective against double resistance[79]. In yet another Spanish report, a 10-d concomitant treatment successfully eradicated 100% and 75% of CAM- and dual-resistant *H. pylori* strains *vs* 75% and 60% respectively with sequential therapy, although the small numbers of dual-resistant strains (4 treated with NBQT, 5 with ST) precludes drawing conclusions[92]. However, a significant clinical impact of dual resistance on the outcome of NBQT was recently showed by Georgopoulos *et al*[93]: including 106 patients with susceptibility testing, eradication rates were

significantly higher in single CAM- and MNZ-resistant strains (100% and 91% respectively) than in dual-resistant *H. pylori* strains (55%), with dual antibiotic resistance remaining as the only predictor of treatment failure by multivariate analysis. Finally, in the most extensive analysis of ST compared to NBQT (evaluating a total of 6 comparative RCTs, 2070 patients), the two regimens performed comparably (81.7% *vs* 81.3%; respectively). However, in 2 out of 6 RCTs, NBQT lasted 5 d only and only one trial was at low risk of bias[80]. Thus, further comparative data is awaited to a definitively address the issue of concomitant *vs* sequential administration. Another promising first-line alternative and a relevant competitor for both ST and NBQT is a two-step hybrid (dual-concomitant) therapy. Originally proposed by Hsu *et al*[94], this treatment yielded high efficacy either against CAM- and dual resistant *H. pylori* strains, demonstrating optimal eradication rates of 97% in ITT and 99% in PP analysis. Evaluation of two RCTs[76,95] comparing hybrid (86.6%) with ST (81%) revealed no statistically significant difference[80]. Similarly, comparison with a 14-d NBQT did not show any significant difference although, interestingly, fewer treatment-related adverse events occurred in those treated with hybrid therapy[90]. Prolonged (14-d) exposure to amoxicillin is likely the key for the improved eradication with hybrid therapy. In line with this hypothesis, no benefit seems to be obtained by prolonging to 14-d duration of ST, in which amoxicillin is discontinued at midpoint[80,96].

Rising prevalence of CAM resistance has prompted use of levofloxacin, a broad spectrum quinolone agent, as a substitute for CAM. A levofloxacin-based triple therapy (LTT) has been proposed as a suitable alternative in settings with a high CAM resistance but < 10% prevalence of levofloxacin resistance achieving 72%-90% cure rates, and even > 95% has been reported provided that 500 mg of levofloxacin *bid* (*vs* 250 mg *bid*) are used[97-99]. Notably, a 5-d levofloxacin-based non-bismuth quadruple regimen was as effective as a 10-d duration same treatment allowing a substantial reduction in treatment costs[100]. In a study by Romano *et al*[101], use of levofloxacin in a 10-d sequential regimen displayed > 95% efficacy (*vs* 80.8% yielded by a CAM-based sequential regimen), although the low levofloxacin resistance rate in this study (3.7%) should be acknowledged. Thus, it may not be feasible to reproduce these good results in most countries, as quinolone resistance currently exceeds 40% in America, 20% in Europe and 10% in Asia[30]. Indeed, rapid development of quinolone resistance has discouraged first-line use of quinolones which are currently reserved as second-line/rescue options after failure of CAM- and/or MNZ-based regimens[102]. In 2006 two meta-analyses confirmed better second-line efficacy with LTT (cure rates 81%-87%) than BQT[103,104]. Similar efficacy (88.7%) was shown by an updated analysis including RCTs up to October 2010, whereas performance of a second-line LTT has been reported to be 74%-81% with stable efficacy over the period 2006-2011 in a Spanish study with 1000 patients[105]. By using LTT after failure of ST (second-line efficacy 75%) Italian investigators obtained a 97.8% of cumulative eradication[106], and the same cure rate (75%) has been reported when using LTT after failure of either first-line ST or NBQT[107]. An impressive 95.8% eradication (ITT, 95%CI: 87.8%-103.8%) was recently reported by using a 10-d quadruple bismuth- and levofloxacin-containing regimen after failure of ST in a small study (24 subjects)[108]. Critically, use of daily levofloxacin doses higher than 500 mg does not seem to confer any therapeutic advantage while it increases the rate of adverse events[109]. Moxifloxacin and Sitafloxacin are newer (and thus more expensive) quinolones which probably encounter less resistance problems. They have shown encouraging results in both first-line and rescue regimens, although no evidence of superiority over levofloxacin is as yet available[110-113].

Susceptibility testing is currently recommended after two consecutive treatment failures. However, when culture of *H. pylori* is not locally available, rifabutin (an anti-tuberculous agent active against *H. pylori*) may be implemented together with a PPI and amoxicillin (all *bid*) as a third-or-more line rescue option providing good results[114]. Using results from 11 studies (2982 patients) the mean rifabutin resistance ranged from 0.6% in treatment-naïve to 1.6% in treatment-experienced patients[115]. Overall, the eradication rates for second- (223 patients), third- (342 patients) and fourth/fifth-line (95 patients) rifabutin-based therapies were 79%, 66% and 70% respectively. Concerning optimal rifabutin dose and treatment duration, most studies recommend 300 mg/d for a total of 10-12 d of treatment. However, clinicians should bear in mind the shortcomings related to rifabutin use, including myelotoxicity and development of resistant *Mycobacterium* species.

## TAILORED TREATMENT OF *H. PYLORI* INFECTION

The continuous rise in prevalence of antibiotic resistance is likely to bring to a deadlock today's efforts to optimize empirical treatments. Even the novel, more effective, empirical regimens discussed above are to some (although lesser as compared to standard therapies) degree prone to the deleterious impact of increasing resistance to their individual key antibiotics. Indeed, results > 95% are infrequently achieved, and even eradication rates > 90% (PP) are disputable[74,77,116]. Tailored therapy of *H. pylori* (as for any other bacterial infection in which near 100% of therapeutic efficacy is obtainable) seems to represent the reasonable next step. This is currently limited by the shortcomings of systematically performing *H. pylori* culture which, as mentioned, is invasive, time-consuming, costly (mainly due to the associated endoscopy costs) and do not 100% reflects in vivo susceptibility[11]. However, molecular testing methods may allow for a rapid and noninvasive characterization *H. pylori* susceptibility to antibiotics revolutionizing our approach to tailored treatment. Nonetheless, a future refinement on tailoring *H. pylori* treatments may be delivered from the field of phar-

macogenomics.

### Culture-guided treatment

Due to the aforementioned limitations of systematically performing culture, this procedure is currently reserved for cases with at least two empirical treatment failures. However, this recommendation has not always yield full consensus among experts. By analyzing 5 RCTs, Wenzhen *et al*[117] showed that a culture-guided triple therapy was more effective and also (based on one RCT focusing on costs) more cost-effective as compared to empirical standard triple therapy. The authors concluded that culture with antimicrobial susceptibility testing should be carried out before first-line treatment. Critically, similar cost-effectiveness evaluations should include the novel BQT, ST and NBQT as, by achieving > 90% and > 80% of first- and second-line efficacy respectively, these regimens are deemed to definitively displace any debate for performing culture systematically.

### Culture-free determination of H. pylori antibiotic susceptibility using molecular methods

Conventional methods to detect macrolide resistance are time-consuming as culture requires 3-10 d and further susceptibility testing (*e.g.*, by Etest, AB bioMerieux; Solna, Sweden) will require additional 3-4 d. These culture-based methods can be now replaced by rapid molecular techniques relying on the measurement of the 3 point mutations in the 23SrRNA gene (namely *A2143G, A2142G* and *A2142C)* which account for 90% of cases of primary CAM resistance in Western countries[25,26]. They include a standard polymerase chain reaction (PCR) and other PCR-based methods including PCR-restriction fragment length polymorphism (RFLP), PCR-DNA enzyme immunoassay (DEIA), PCR oligonucleotide ligation assay (OLA) and PCR-line probe assay[118,119]. Real-time PCR assays, representing a powerful advancement of the basic PCR method, have been also developed and are commercially available[120]. These PCR-based methods can be directly applied on gastric specimens, offering fast and highly accurate results (reportedly > 80%-90% of both sensitivity and specificity) including detection of the heteroresistant status (defined as the co-existence of strains susceptible and resistant to the same antibiotic in the same patient), which is not detectable by conventional culture-based methods accounting for a significant rate of treatment failures[28,121,122]. Importantly, no additional gastric mucosal specimens are required as samples stored in rapid urease test, in room temperature, for up to 30 d, may be successfully used for PCR obviating the necessity to freeze specimens for off-site testing[123,124]. Genotypic detection of macrolide resistance without PCR is also possible by using fluorescence *in-situ* hybridization (FISH). It uses fluorescent probes specific to mutations associated with resistance and has the advantage that it can be applied on paraffin-embedded tissue samples, thus it can be offered by pathology services[122,125,126]. A great application of molecular methods is non-invasive (*i.e.*, without need for endoscopy) determination of *H. pylori* susceptibility to antibiotics. Indeed, minimally-invasive techniques (including oro-gastric brushing and gastric wash) or use of stool specimens can be used to obtain *H. pylori* DNA for molecular testing[127-130]. Few available studies have assessed the benefits of a PCR-based tailoring of treatment for *H. pylori* infection. In a Japanese study, tailored treatment using a dual PPI/MNZ regimen for CAM-resistant strains yielded 94.3% eradication (100% against CAM-resistant strains) *vs* 71.4% using empirical standard treatment[127]. In a larger Asian RCT, 218 patients were treated with a PCR-tailored triple therapy: if a CAM-resistant strain was detected, then CAM was replaced by MNZ in the triple regimen[131]. Eradication rates were 91.2% in the tailored group *vs* 79.1% and 75.9% by using empirical MNZ- and CAM-based triple therapies ($n$ = 308 in each control group) respectively ($P < 0.001$). A molecular approach is also available to test for levofloxacin resistance based on the detections of *gyrA* mutations[132]. Contrarily, there is not such availability for MNZ resistance as molecular basis of that phenomenon remain not fully understood.

### Pharmacogenomic-tailored therapy

Apart from bacterial susceptibility to antimicrobial agents, magnitude of the acid suppression achieved by PPI's is critical for the treatment of *H. pylori* infection; thus, an ideal tailored treatment should also target to optimize the PPI's metabolism[133,134]. PPI's are metabolized by cytochrome P450 (CYP) 2C19 (CYP2C19) in the liver[135]. However, there is substantial genetic variability in the activity of this enzyme. Three different CYP2C19 genotypes are recognized, influencing the PPI plasma levels and consequently the eradication of *H. pylori*: rapid metabolizer, intermediate metabolizer and poor metabolizer, with plasma PPI levels and intragastric pH's being lowest in the rapid metabolizer group[136]. In a landmark study, 300 *H. pylori*-positive patients were randomized to either a 1 week standard regimen or to personalized therapy based on both CYP2C19 and CAM susceptibility status assessed by genetic testing[137]. The ITT eradication rates were significantly higher in the tailored group (96% *vs* 70%) without an increase of the final per-patient cost for successful eradication. Critically, in this study lansoprazole was used, which is known to be affected by CYP2C19 status[138]. In contrast, the metabolism of other PPI's (*e.g.,* esomeprazole or rabeprazole) has been reported to be independent to CYP2C19 status[139,140], thus choice of these agents could probably outplace the need for CYP2C19 genotyping. Further studies are warranted to clarify the role of PPI pharmacogenomics as a basis for tailoring *H. pylori* eradication therapies.

## PROBIOTICS

Adjunct use of probiotics has attracted growing attention in recent years as a strategy aimed to both improve eradication rates and prevent occurrence of *H. pylori*

therapy-related side-effects. Although mechanisms of a possible inhibitory effect of probiotics on *H. pylori* remain largely unknown, some hypothesis have been put forward including production of an inhibitory substance, competition for adhesion, strength of the mucosal barrier, and modulation of the *H. pylori*-related immune cascade of the host[141]. To date, several trials supported co-administration of probiotics together with standard[142], sequential[143] or levofloxacin-based regimens[144], whereas others did not[145-147]. Both single-strain (mostly *Lactobacillus* spp., *Saccharomyces* spp., *Bifidobacterium* spp. and *B. clausii*) and multistrain[146] compounds have been evaluated. Lack of placebo control, and a substantial heterogeneity in treatment duration as well as choice of different probiotic strains may have accounted for the conflicting results between studies. In a systematic review, Wilhelm *et al*[148] concluded that probiotics may be beneficial in reducing adverse effects and increase tolerability of *H. pylori* eradication regimens, particularly in cases with recurrent infection or history of gastrointestinal antibiotic-related side-effects. To date, meta-analytic evidence has been provided for using either *Saccharomyces boulardi* (OR = 1.13, 95%CI: 1.05-1.21) or *Lactobacillus* spp. (OR = 1.78, 95%CI: 1.2-2.6) supplementation adjunctively to standard triple therapy[149,150]. A recent meta-analysis (10 trials, 1469 patients) assessed the effect of Lactobacillus-containing and Bifidobacterium-containing supplementation during *H. pylori* therapy: the pooled odds ratio (ITT) with probiotic supplementation was 2.066 (95%CI: 1.398-3.055) for eradication and 0.305 (95%CI: 0.117-0.793) for the incidence of total side effects[151]. In conclusion, although increasing evidence supports probiotic supplementation, further studies are required to better characterize the magnitude and mechanisms of a beneficial effect of probiotics, standardize administration, and assess cost-effectiveness, as these agents are not inexpensive.

## CONCLUSION

Due to the wide-spread consumption of antibiotics, *H. pylori* resistance to CAM is now exceeding 20% in many parts of the world. Conventional triple therapies, which for many years have represented the backbone of treating *H. pylori*, are not any more recommended for empiric use, and should not be prescribed, unless local CAM resistance is low or culture confirms susceptibility to CAM. More effective CAM-based regimens are now replacing standard triple therapies as empirical first-line treatments. These include the sequential, non-bismuth quadruple (concomitant) and dual-concomitant (hybrid) regimens. A bismuth-containing quadruple regimen can be effectively used either as first-line or rescue option when a CAM-based regimen fails. Rapidly growing quinolone resistance has precluded use of levofloxacin in first-line treatments. Thus, substitution of CAM with levofloxacin in triple, sequential or quadruple regimens should be reserved as a second-line/rescue option when CAM- and/or MNZ-based regimens fail. Critically, due to the steady increase in prevalence of antimicrobial resistance, empiric use of either CAM or levofloxacin may become no longer feasible in the future. Tailored treatment of *H. pylori* infection appears as the reasonable alternative to maintain high therapeutic efficacy, thus avoiding exposing the patient to repeated empirical antibiotic courses. A culture-based tailoring of therapy is currently recommended after at least two empirical treatment failures as it has obvious limitations, including it is invasive, time-consuming, and costly. Molecular PCR-based and FISH methods may allow for a rapid, non-invasive and highly accurate determination of *H. pylori* susceptibility to antibiotics, including detection of the heteroresistant status. Tailoring treatment according to the CYP2C19 status affecting the metabolism of PPIs may represent a further refinement delivered by the field of pharmacogenomics. Both practical and logistic issues should be addressed before a tailored approach based on the genotypic detection of *H. pylori* therapeutic susceptibility is ready for wide-spread implementation into routine practice. Until then, efforts to enhance performance of empirical treatments should continue, including use of probiotics in the therapeutic schemes.

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TOPIC HIGHLIGHT

WJGP 5th Anniversary Special Issues (1): *Helicobacter pylori*

# Treatment of *Helicobacter pylori* infection: Past, present and future

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## Abstract

*Helicobacter pylori* (*H. pylori*) is a major human pathogen associated with significant morbidity and mortality. However, after decades of efforts, treatment of *H. pylori* remains a challenge for physicians, as there is no universally effective regimen. Due to the rising prevalence of antimicrobial resistance, mainly to clarithromycin, efficacy of standard triple therapies has declined to unacceptably low levels in most parts of the world. Novel regimens, specifically experimented to improve the therapeutic outcome against antibiotic-resistant *H. pylori* strains, are now recommended as first-line empirical treatment options providing high efficacy (reportedly > 90% in intention to treat analysis) even in high clarithromycin resistance settings. These include the bismuth quadruple, concomitant, sequential and hybrid therapies. Due to the rapid development of quinolone resistance, levofloxacin-based regimens should be reserved as second-line/rescue options. Adjunct use of probiotics has been proposed in order to boost eradication rates and decrease occurrence of treatment-related side effects. Molecular testing methods are currently available for the characterization of *H. pylori* therapeutic susceptibility, including genotypic detection of macrolide resistance and evaluation of the cytochrome P450 2C19 status known to affect the metabolism of proton pump inhibitors. In the future, use of these techniques may allow for culture-free, non-invasive tailoring of therapy for *H. pylori* infection.



**Key words:** *Helicobacter pylori*; Antibiotic resistance; Bismuth-quadruple; Concomitant; Sequential; Probiotics

**Core tip:** Worldwide increase in prevalence of macrolide resistance has accounted for the failure of standard therapies for the treatment of *Helicobacter pylori* (*H. pylori*) infection. Bismuth quadruple, concomitant, sequential and hybrid therapies are now recommended as first-line empirical treatments providing improved efficacy in high clarithromycin resistance settings. As quinolone resistance is rapidly increasing, levofloxacin should be preferentially used in second-line/rescue therapies. There is increasing evidence that adjunct probiotic supplementation improves the therapeutic outcome and tolerability. Genotypic characterization of *H. pylori* susceptibility to therapy may allow for a tailored therapeutic approach in the future.

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## INTRODUCTION

Treatment of *Helicobacter pylori* (*H. pylori*) infection is paramount for the management of prevalent gastrointestinal

**Table 1 Factors reported to negatively affect the outcome of therapies for *Helicobacter pylori* infection**

| Pathogen-related | Host-related |
|---|---|
| Development of resistance to antibiotics | Non-compliance to treatment |
| High bacterial load in the stomach | Non-ulcer dyspepsia |
| Protective effect of the gastric mucus layer | Smoking |
| Intracellular location of many bacteria | CYP2C19 status (rapid metabolizer) |
| *CagA* negative | |
| Presence of dormant coccoid forms (not susceptible to antibiotics) | |
| Heteroresistant status (co-existence of strains susceptible and resistant to the same antibiotic) | |

diseases, including peptic ulcer disease, gastric cancer and functional dyspepsia[1-3]. Moreover, extra-digestive disorders are now included as indications for eradication of *H. pylori*: idiopathic thrombocytopenic purpura, vitamin B12 deficiency and unexplained iron deficiency anemia[4]. Contrarily to other bacterial infections, for which susceptibility testing is commonly performed to guide treatment, culture of *H. pylori* is not widely available and requires performing endoscopy which is not well-tolerated by all patients and has a series of limitations, including the fact that *in vitro* susceptibility does not always guarantee *in vivo* eradication[5]. Hence, regimens for *H. pylori* have been routinely prescribed empirically, provided they have been previously tested and sufficiently tailored with regard to various parameters (*i.e.*; treatment dose, duration, dosing intervals *etc.*) to optimize cure rates and minimize side effects. However, the optimal treatment to eradicate *H. pylori* remains to be established, as no regimen is effective universally. Worldwide increase in resistance to key antibiotics, mainly clarithromycin (CAM), but also metronidazole (MNZ) and levofloxacin, is the main determinant of failure in the treatment of *H. pylori* infection[6,7]. In a recent systematic review, the global incidence of CAM resistance has been reported to be 17.2% ranging from 11.1% in Europe to 29.3% in America, whereas, in the same analysis, continental rates of resistance to MNZ were 17% and 44.1% respectively[8]. Antibiotic consumption for infections other than *H. pylori* is accounting for the wide increase in *H. pylori* antibiotic resistance rates[9,10]. Indeed, different national policies for antibiotic use are largely reflecting geographical distribution of *H. pylori* resistance: CAM resistance has been reported to be significantly higher in Southern European countries (reaching 49% in some areas of Spain) as compared to Northern Europe (*e.g.,* only 1% in the Netherlands) where policies for antibiotic use are more stringent[9]. Additionally to the development of antibiotic resistance, a series of both host and pathogen related factors may negatively impact on the performance of regimens to eradicate *H. pylori* (Table 1)[11,12].

Despite decades of efforts, treatment of *H. pylori* infection remains a challenging issue for both researchers and practicing physicians. In the present article we aim to provide a comprehensive overview of perspectives on the past, present and future of *H. pylori* eradication.

## CLARITHROMYCIN-BASED TRIPLE THERAPIES: A DECLINING CLINICAL STANDARD

Historically, the first truly effective therapy for *H. pylori* infection, comprising of bismuth, tetracycline and MNZ, was proposed in 1990[13]. A few years later, use of CAM in a triple therapy, proposed by Bazzoli *et al*[14], was the start of CAM-based triple regimens, thereafter representing the gold standard in the treatment of *H. pylori*. In studies conducted during the 90's, standard triple therapies (STT) comprising of a proton pump inhibitor (PPI) *bid*, CAM 500 mg *bid* and amoxicillin 1000 mg *bid* or MNZ 500 mg (or 400 mg in England), all given for 7-14 d, provided consistently good results yielding > 80% eradication success and even > 90% was feasible[15,16]. Due to this high efficacy and relative simplicity, optimal safety profile, and large pharmaceutical company commitment, these regimens have been widely accepted in national expert panels and consensus recommendations worldwide as standard of care treatments for first-line eradication of *H. pylori*[17-20]. However, rising prevalence of CAM resistance has accounted for a significant decline in the efficacy of standard regimens. This decreasing efficacy was already evident in the meta-analyses published by the early 2000's, prompting significant changes in the paradigm of treating the infection. These included the introduction of the concept of cumulative treatment efficacy (requiring the patient to comply with more treatment courses; thus, more side effects and spreading of secondary antibiotic resistance), and later the introduction of a local threshold (15%-20%) of CAM resistance at which CAM should not be used empirically[17,18]. The decreased efficacy of standard treatments against CAM-resistant strains has been well-documented on a meta-analytic basis: In a meta-analysis by Fischbach and Evans, the success of triple therapy was decreased by 66.2% (95%CI: 58.2%-74.2%) when strains of *H. pylori* were resistant *vs* susceptible to CAM[7]. Congruently, a more recent analysis by Venerito *et al*[21], revealed similar results: including antimicrobial susceptibility data from 4 randomized clinical trials (RCTs), standard triple therapies successfully eradicated 88% of CAM-sensitive but only 14% of CAM-resistant *H. pylori* strains (risk difference = 0.75, 95%CI: 0.63-0.87). If MNZ is used, presence of MNZ resistance may also affect the therapeutic outcome[22], although it is generally considered less important clinically. This is due to the fact that MNZ resistance may be largely overcome by increasing dose and prolonging treatment duration[23]. Lastly, *H. pylori* resistance to amoxicillin is exceptional and generally is not relevant clinically. In the light of increasing data confirming suboptimal performance (< 70%) in most

**Table 2 Current regimens to treat *Helicobacter pylori* infection**

| Treatment | Regimen |
|---|---|
| Bismuth-containing quadruple therapy | A PPI (standard dose, *bid*), bismuth (standard dose, *qid*) tetracycline (500 mg, *qid*) and metronidazole (500 mg, *qid*) for 10-14 d |
| Sequential therapy | A 5-d dual therapy with a PPI (standard dose, *bid*) and amoxicillin (1 g, *bid*) followed by a 5-d triple therapy with a PPI (standard dose, *bid*), clarithromycin (500 mg, *bid*) and metronidazole (500 mg, *bid*) |
| Concomitant therapy | A PPI (standard dose, *bid*), clarithromycin (500 mg, *bid*), amoxicillin (1 g, *bid*) and metronidazole (500 mg, *bid*) for 7-10 d |
| Hybrid therapy | A 7-d dual therapy with a PPI (standard dose, *bid*) and amoxicillin (1 g, *bid*) followed by a 7-d quadruple therapy with a PPI (standard dose, *bid*), amoxicillin (1 g, *bid*), clarithromycin (500 mg, *bid*) and metronidazole (500 mg, *bid*) |
| Levofloxacin-based triple therapy | A PPI (standard dose, *bid*), levofloxacin (500 mg, *bid*) and amoxicillin (1 g, *bid*) for 10 d |

PPI: Proton pump inhibitor.

European countries, the recent Maastricht IV/ Florence consensus report has definitively displaced standard regimens as the empirical gold standard to eradicate *H. pylori*[4]. Instead, use of legacy triple regimens should take into account the local resistance pattern (thus, used only in areas in which CAM resistance is < 20%) or rely on susceptibility testing provided that pre-treatment culture is available (*i.e.*, used as tailored treatments).

## CURRENT THERAPIES FOR *H. PYLORI* INFECTION

Novel regimens, specifically experimented to improve the therapeutic outcome against antibiotic-resistant *H. pylori* strains, are now recommended as first-line empirical treatment options providing improved efficacy (reportedly > 90% in intention to treat analysis) in high CAM resistance settings. These regimens are summarized in Table 2.

## BISMUTH QUADRUPLE THERAPY

Bismuth quadruple therapy (BQT) currently represents a preferred first-line treatments option for areas with a high (≥ 20%) incidence of CAM resistance but also a valuable second-line treatment option when a CAM-based regimen has previously failed. It works independently to CAM achieving > 90% eradication in the presence of CAM resistance, whereas implementation of a high MNZ dose (1500-1600 mg/d) and prolonged (10-14 d) treatment duration allow for minimizing the impact on MNZ resistance, providing eradication rates > 85% even in regions with a high resistance to this drug[24]. A patient-friendly monocapsule (containing bismuth, MNZ and tetracycline) is available (Pylera®, Aptalis, Mont St Hilaire, QC, Canada) providing intention-to-treat eradication rates of 86% and 80% in two large RCTs conducted in North America and Europe respectively[25-27]. Contrarily, the ITT eradication rate with BQT was only 77.8% in a recent meta-analysis (*vs* 77% for STT), questioning both the efficacy as well as the superiority of the BQT over STT[28]. However, a substantial grade of study heterogeneity, especially with respect to MNZ dosing, should be acknowledged. The second-line efficacy of BQT has been also confirmed on a meta-analytic basis (30 studies) showing an average 77% second-line efficacy (ITT) after failure of STT[29]. Third-line efficacy of BQT after two previous eradication failures with CAM- and levofloxacin-containing triple therapies was 65% (ITT) in a multi-center study from Spain[30]. Non-availability of bismuth salts or tetracycline in some countries as well as the potential toxicity of bismuth are the main limitations. However, including 4763 patients no differences with respect to tolerability were shown between non-bismuth and bismuth-containing groups except from dark stools being more common in the later[31].

## SEQUENTIAL THERAPY

Sequential therapy uses the same antibiotics contained in STT but administered sequentially. It has been postulated that the initial course of amoxicillin disrupts the bacterial cell wall preventing the development of efflux channels transferring CAM out of the bacteria[32]. Although in the initial RCTs[33] (most of them conducted in Italy) and earlier meta-analyses sequential therapy was clearly superior to STT [ITT eradication 91.7% (95%CI: 90%-93%) *vs* 76.7% (95%CI: 75%-79%) for STT][34], more recent data from South America, Iran and South Korea revealed lower eradication rates (< 80%)[35-37]. Despite this sequential therapy seems to be fairly effective against CAM mono-resistant strains, being able to eradicate 72.8% of them, its efficacy against dual resistant (CAM and MNZ) strains was only 37% (range: 16.2% to 60.7%) when 8 studies with antibiotic susceptibility data were evaluated[38]. Critically, sequential therapy was not superior to either a 14-d triple therapy (RR = 1, 95%CI: 0.94-1.06) or a bismuth-based therapy (RR = 0.99, 95%CI: 0.94-1.05) in an extensive evaluation of 46 RCTs[38].

## NON-BISMUTH QUADRUPLE (CONCOMITANT) THERAPY

A non-bismuth quadruple "concomitant" therapy is another valid first-line treatment option for areas with a high incidence of CAM resistance[39,40]. In 19 studies (2070 patients) the overall eradication rate with concomitant therapy was 88% (95%CI: 85%-91%) and 91% when 3 outlying studies with inherently short treatment duration (3-5 d) were excluded[41]. Indeed, treatment duration of

at least 7 d has been shown to be necessary for the success of concomitant therapy[42], whereas extra-prolonging treatment to 14 d combined with a high PPI dose (omeprazole 40 mg × 2) may further boost cure rates to > 95%, as revealed by a non-inferiority multi-center trial[43]. An increased efficacy against dual resistant *H. pylori* strains has been proposed as the main strength of the concomitant over the sequential therapy[44], though the two regimens have performed equally when compared using 338 patients in a high antibiotic resistance country (Spain)[45]. Indeed, by evaluating 106 patients with pre-treatment susceptibility testing, the concomitant therapy eradicated only 55% of dual-resistant strains *vs* 100% and 91% with CAM and MNZ resistance respectively[46]. Thus, both regimens seem to be prone to the deleterious impact of dual resistance, performing comparably (with about 81% of efficacy each) by pooling data of 6 comparative RCTs[38].

## HYBRID THERAPY

A two-step dual-concomitant (hybrid) regimen, proposed by Hsu *et al*[47], is another valuable treatment option competing with both the sequential and concomitant treatments. By evaluating data from 2 RCTs, hybrid therapy performed marginally, though not significantly, better as compared to sequential therapy (86.6% *vs* 81%)[38], and comparably to concomitant therapy in a comparative study in which, interestingly, fewer adverse events occurred in the group treated with the hybrid regimen[43]. Further data is warranted to allow for definitive conclusions on the efficacy and tolerability of hybrid therapy.

## LEVOFLOXACIN-BASED THERAPIES

To overcome increasing CAM resistance, levofloxacin, a broad spectrum quinolone, has been used as a substitute of CAM in either triple or sequential regimens achieving > 90% cure rates, and even > 95% is feasible provided that the local resistance to levofloxacin is low (< 10%)[48,49]. However, levofloxacin also encounters clinically significant problems of antibiotic resistance, as resistance to quinolones currently exceeds 40% in America, 20% in Europe and 10% in Asia[8]. Due to the rapid development of secondary quinolone resistance, first-line use of levofloxacin is generally discouraged, and the drug is reserved for use in second-line/rescue regimens after failure of a CAM- and/or a MNZ-based regimen[50]. The good (cure rates 81%-87%) second-line efficacy of a levofloxacin triple therapy (LTT) has been confirmed in two meta-analyses published in 2006, both showing better results with LTT in comparison with second-line BQT[51,52]. Congruently, second-line efficacy of LTT was 88.7% in a more recent meta-analysis including RCTs up to October 2010[53]. Critically, use of LTT after failure of either a sequential or concomitant regimen has been reported to provide up to 97.8% of cumulative therapeutic efficacy[54]. Use of other quinolone agents, such as Moxifloxacin and Sitafloxacin, has shown promising results[55,56], though there is no evidence to support any therapeutic advantage over levofloxacin.

## FUTURE PERSPECTIVES

### Adjunct probiotics

Albeit different attempts have been made to restore the efficacy of standard treatments, such as increasing the PPI dose or prolonging treatment duration, none has been proved at a level to overcome today's antimicrobial resistance. An approach which has attracted growing interest is using probiotics in conjunction with regimens to eradicate *H. pylori*[57]. The expected benefit is twofold: boosting eradication and improving tolerability by preventing occurrence of treatment-related side effects. The pathogenic basis of a possible beneficial effect of probiotics on *H. pylori* eradication remains to be clarified, though some hypothesis have been put forward including strength of the mucosal barrier, competition for adhesion and immunomodulatory mechanisms[58]. Different trials used probiotics adjunctively to either standard or novel regimens in recent years providing contradictory results[59-62]. Although different single- or multi-strain compounds have been evaluated, there is currently evidence to support use of *Saccharomyces boulardi* (OR = 1.13; 95%CI: 1.05-1.21) or *Lactobacillus spp.* (OR = 1.78; 95%CI: 1.2-2.6) supplementation adjunctively to standard triple therapy[63,64]. In the most recent analysis assessing the effect of Lactobacillus-containing and Bifidobacterium-containing supplementation, the pooled odds ratio (ITT) with probiotic supplementation was 2.066 (95%CI: 1.398-3.055) for eradication and 0.305 (95%CI: 0.117-0.793) for the incidence of total side effects[65]. Interestingly, with respect to the prevention of side-effects, use of probiotics may be relevant only in a subset of patients, in particularly those with recurrent infection or history of gastrointestinal antibiotic-related side effects[57]. Further data is awaited to clarify the role, standardize regimens and assess the cost-effectiveness of probiotics in the treatment of *H. pylori* infection.

### Culture-free, non-invasive determination of H. pylori antibiotic susceptibility

Critically, even the novel treatments discussed above are to some (although to a lesser as compared to legacy therapies) degree prone to the impact of antibiotic resistance; eradication rates > 95% are infrequent, and even > 90% are disputed in some studies[35,66,67]. Furthermore, it is possible that the success of empirical treatments will further decline in the future as resistance to key antibiotics is constantly growing worldwide. In order to maintain high therapeutic efficacy, tailored treatment of *H. pylori* infection based on pre-treatment susceptibility testing appears as the ideal approach. This will prevent exposing the patient to repeated empirical treatments which increase the risk for side effects and promote development of secondary resistance. However, as

mentioned, current means of performing endoscopy and *H. pylori* culture are invasive, do not 100% reflect in vivo susceptibility, and are time-consuming as culture requires 3-10 d and susceptibility testing (*e.g.*, by Etest, AB bioMerieux, Solna, Sweden) will require additional 3-4 d. These limitations preclude systematical performance of *H. pylori* culture, which is currently recommended only for cases with at least two empirical treatment failures. A class-wide resistance to macrolides is the result of point mutations in three adjacent nucleotide positions (*A2143G, A2142G and A2142C*) in the peptidyl transferase loop of the *23SrRNA* gene[68,69]. These three point mutations account for 90% of cases of primary CAM resistance in Western countries. In recent years, molecular testing methods have been developed for these mutations including a standard polymerase chain reaction (PCR) and other PCR-based methods including PCR-restriction fragment length polymorphism, PCR-DNA enzyme immunoassay, PCR oligonucleotide ligation assay and PCR-line probe assay, as well as Real-time PCR assay which represents a powerful advancement of the basic PCR[70-72]. These methods may offer rapid and highly accurate results in the genotypic detection of CAM resistance, including detection of the heteroresistant status (*i.e.*, co-existence of strains susceptible and resistant to the same antibiotic) known to account for a significant number of treatment failures[73,74]. These techniques can be directly applied on gastric biopsy specimens or used in association with minimally-invasive techniques (*e.g.,* oro-gastric brushing or gastric wash) or non-invasively using stool specimens[75-77]. Importantly, genotypic detection of CAM resistance is also possible with Fluorescence In-Situ Hybridization, which can be also applied on paraffin-embedded specimens[78,79]. Detection of levofloxacin resistance based on the detection of *gyrA* mutations is also available[80]. Two Asian studies have provided data on the potential utility of a tailored therapeutic approach based on the molecular detection of *H. pylori* resistance to CAM. Tailored treatment using a simple PPI/MNZ regimen successfully eradicated the pathogen in 94.3% *vs* 71.4% using empirical standard treatment[77]. In a larger study (218 patients), CAM was replaced by MNZ in the triple regimen if a CAM-resistant strain was detected. Eradication rates were 91.2% in the tailored group *vs* 79.1% and 75.9% by using empirical MNZ- and CAM-based triple therapies ($n$ = 308 in each control group) respectively ($P < 0.001$)[81].

### Pharmacogenomics

Genetic variability in the activity of the cytochrome P450 (CYP) 2C19 (CYP2C19) is known to influence the plasma levels of PPIs, and thus treatment of *H. pylori* infection[82,83]. Three distinct genotypes are recognized: rapid, intermediate and poor metabolizers. Preliminary data on the potential use of pharmacogenomics has been provided by a RCT with 300 *H. pylori*-positive patients randomized to either a 1 wk standard regimen or to personalized therapy based on both CYP2C19 and CAM susceptibility status assessed by genetic testing[84]. The ITT eradication rates were significantly higher in the tailored group (96% *vs* 70%) without an increase of the final per-patient cost for successful eradication. In the future, both practical and logistic issues should be addressed before a molecular-based approach can be widely adopted as a genuine basis for the individualization of *H. pylori* eradication therapies.

## CONCLUSION

For more than a decade, triple regimens have been the standard of care therapies for *H. pylori* infection. However, in more recent years, rising prevalence of macrolide resistance has accounted for a significant decline in the performance of these regimens, resulting in the necessity of more treatment courses in order to eradicate the pathogen. In order to maintain high therapeutic efficacy, regimens with an improved performance against antibiotic-resistant *H. pylori* strains are now recommended as preferred first-line treatments. The concomitant and sequential regimens are currently the best validated first-line therapeutic options. Hybrid therapy is another effective CAM-based alternative and a relevant competitor to both these treatments. BQT is also a valid treatment for high CAM resistance settings, but also an effective second-line regimen when a CAM-based regimen fails. Due to the rapid development of quinolone resistance, levofloxacin-based regimens should be currently reserved as second-or-more-line treatment options. While efforts to improve empirical treatments continue, the fields of genotypic detection of *H. pylori* antimicrobial susceptibility and pharmacogenomics offer a fascinating new perspective. This is to guarantee 100% therapeutic efficacy: fast, culture-free and non-invasive.

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TOPIC HIGHLIGHT

WJG 20th Anniversary Special Issues (6): *Helicobacter pylori*

# *Helicobacter pylori* infection and inflammatory bowel disease: Is there a link?

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## Abstract

*Helicobacter pylori* (*H. pylori*) infection is one of the most widely spread infectious diseases in humans. It can cause chronic gastritis, peptic ulcer disease and gastric malignancies and has been associated with extra-gastric disorders. *H. pylori* elicit a chronic systemic inflammatory response which, under certain conditions, may trigger autoimmune reactions and may be implicated in the pathogenesis of autoimmune diseases. Although the pathogenesis of inflammatory bowel disease (IBD) is unknown, it is thought to result from complex interactions between environmental factors and microbiota in the gut of individuals who are genetically susceptible. Several bacterial and viral agents have been implicated in the aetiology of IBD. In theory, *H. pylori* infection could be involved in the pathogenesis of IBD by inducing alterations in gastric and/or intestinal permeability or by causing immunological derangements resulting in absorption of antigenic material and autoimmunity via various immunological pathways. Similar mechanisms may also be responsible for the co-existence of IBD with other autoimmune diseases and/or extra-intestinal manifestations. However, the epidemiological data fail to support this association. In fact, various studies indicate that the prevalence of *H. pylori* infection is low in patients with IBD, suggesting a protective role for this infection in the development of IBD. In this report, we aim to shed light on proposed mechanisms and confounding factors underlying the potential link between *H. pylori* infection and IBD.



**Key words:** *Helicobacter pylori*; Inflammatory bowel disease; Ulcerative colitis; Crohn's disease; Colorectal cancer

**Core tip:** By gathering a large volume of published data, this review attempts to shed light on the mechanisms and confounding factors underlying the potential link between *Helicobacter pylori* (*H. pylori*) infection and Inflammatory Bowel Disease (IBD). However, whether the link between *H. pylori* and IBD is coincidental, epiphenomenal or mechanistic remains to be elucidated as there are contradictory data regarding both the causative and the protective role of *H. pylori* infection against IBD. This review provides a tool for researchers in this field to use as they perform further research to find the missing links.

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## INTRODUCTION

Inflammatory bowel diseases (IBDs), which includes Crohn's disease (CD) and ulcerative colitis (UC), are chronic, relapsing-remitting diseases that constitute a growing worldwide health burden[1-3]. Over time, these

diseases may lead to intestinal damage, complications, surgical interventions, gut failure and/or disability[4-7]. IBD is thought to result from complex and unidentified interactions between environmental factors (such as infections, medicines, tobacco, food particles) and genetic factors of the host, resulting in abnormal and/or inappropriate immunological reactions to elements of the intestinal flora. For example, Gradel *et al*[8] demonstrated that infection with either *Campylobacter* or *Salmonella* species predisposed individuals to subsequent development of IBD.

*Helicobacter* species easily colonize the gastrointestinal surface due to microaerophilic metabolism, spiral shape, and peculiar motility[9]. Based on their location within the gastrointestinal system, they are divided into gastric *Helicobacters,* such as the *Helicobacter pylori* (*H. pylori*), and enterohepatic *Helicobacters* (EHH), which predominantly colonize the intestine and the hepato-biliary system and have been linked to chronic liver and intestinal diseases[9]. *H. pylori* usually resides in the surface epithelium of the stomach, but *H. pylori* DNA has also been identified in both the colon[10] and stool of infected patients[11-13].

*H. pylori* is a gram-negative, spiral-shaped pathogenic bacterium that causes chronic gastritis. Peptic ulcer disease and/or gastric malignancies may develop in a small number of individuals infected with the bacterium[9,14]. The inflammatory response of the gastric mucosa to *H. pylori* most likely reflects the combined effects of a cellular immune response that is driven by an on-going stimulation of the host's immune system by the bacterium. This results in high production of interleukin (IL)-12, leading to a T helper type 1 (Th1)-polarized response and elevated levels of Th1 cytokines[15-18]. Products of the local immune reactions may travel to extra-gastric sites, thus linking *H. pylori* infection to the pathophysiology of a variety of extra-gastric diseases, including autoimmune disorders[19-21]. Interestingly, however, *H. pylori* has been proposed to play a protective role against the development of certain autoimmune disorders[21] such as asthma[22] and type 1 diabetes mellitus[23]. The mechanisms underlying this protective role of *H. pylori* infection is thought to be differential expression of an acute and/or chronic local mucosal inflammatory response, which may elicit a systemic release of cytokines[24], which in turn may down-regulate systemic immune responses and suppress autoimmunity.

In IBD, dysregulation of the immune response of the host to commensal bacteria has been proposed as an important underlying pathogenetic mechanism. Increased attachment of gut bacteria to the intestinal epithelium has been documented in IBD. A Th1 immune reaction and secretion of pro-inflammatory cytokines is implicated in the pathogenesis of IBD, especially CD[5]. Up-regulation of cell signalling molecules, such as the macrophage inflammatory protein 3a (MIP-3a), has also been documented in IBD[25]. Some IBD patients suffer from concomitant autoimmune diseases and autoimmune-type extra-intestinal manifestations. The similarities between the immunobiology of IBD and that of *H. pylori* infection provides background for the hypothesis that *H. pylori* infection may be implicated in the pathogenesis of IBD.

Nevertheless, there is epidemiological evidence that contradicts the association between *H. pylori* and IBD. *H. pylori* infection is an infection that occurs in underprivileged societies and its prevalence declines when environmental hygienic conditions improve. In contrast the prevalence of IBD increases in societies adapting a Western life style[26]. Thus, it appears that there is an inverse relation between the prevalence of IBD and *H. pylori* infection. IBD is highly prevalent in the United States[27], an area with low rates of *H. pylori* infection whereas, a steady rise in the incidence of IBD has been observed in *H. pylori* endemic regions following widespread use of therapeutic regimens to treat *H. pylori*[28]. Although environmental changes may be the confounding factor underlying this inverse relationship, many (but not all) epidemiological studies have shown a low incidence of *H. pylori* infection in patients with IBD[29-60]. This has led to the hypothesis that *H. pylori* infection may exert a protective role against IBD. However one can argue that it is the medication used to treat IBD that eradicates *H. pylori* and /or the IBD associated mucosal alterations that may prevent colonization of the stomach by *H. pylori*. The latter may be true, especially in IBD patients with focally enhanced gastritis (FEG) who have a particularly low incidence of *H. pylori* infection, even if they live in *H. pylori* endemic areas[33,34,61-64].

Possible mechanisms of the potential protective role of *H. pyloric* infection against the development of IBD may be alteration of the host immunologic response away from the pro-inflammatory Th1/Th17 response towards an increased T-regulatory cell immune response[65,66]. Moreover, *H. pylori* may induce the production of antibacterial peptides that counteract potentially harmful bacteria implicated in the pathogenesis of IBD[67] or compete with bacteria for the same ecologic niche in the upper gastrointestinal tract[68].

## CAUSAL ASSOCIATION OF *HELICOBACTER* SPECIES AND *H. PYLORI* WITH IBD

In animal models, EHH such as *Helicobacter hepaticus* (*H. hepaticus*) and *Helicobacter bilis* (*H. bilis*) have been shown to induce a persistent inflammation in the colon and cecum in immuno-deficient rodents[69,70]. *Helicobacter hepaticus* triggers colitis in a specific pathogen-free IL-10-deficient mice through an IL-12 and interferon-gamma (IFN-γ) dependent mechanism[69]. *Helicobacter muridarum* increases disease activity and inflammation in an acute colitis model[71] and provokes a CD-like inflammation in severe combined immunodeficiency mice upon receipt of T cells[72]. Accumulating evidence from gene knockout rodents also indicate that the presence of EHH worsens the severity or hastens the development of colitis[73,74].

However, observations from human studies are conflicting. Several EHH species have been identified in the

large intestine of patients with enteritis and / or proctitis[75]. *Helicobacter macacae* has been linked with chronic idiopathic colitis in young rhesus monkeys[76]. Similarly, Laharie *et al*[77] found that *Helicobacter pullorum* (*H. pullorum*) or *Helicobacter canadensis* infection was significantly associated with CD in adults. *Helicobacter* species were found either in faecal specimens[78] or in colonic biopsy samples[79] of children with CD, and the prevalence of the *Helicobacteraceae* was significantly higher in children with CD (32/77, 41.5%) compared to controls (23/102, 22.5%)[80]. A German group found *Helicobacter fennelliae* and *H. pullorum* in colonic samples of 12% of CD patients[81]. *Helicobacter* genus PCR positivity was also significantly higher in UC than in controls (32/77 *vs* 11/59, $P = 0.004$)[82]. *H. pylori* was isolated and detected by PCR in the intestinal mucosa of patients with UC-like CD and UC[53,54,83]. Moreover, in another study *H. pylori* was found in faecal specimens in the majority of children with CD[78]. In contrast, *Helicobacter* species were not detected in colonic biopsies of IBD patients in various studies[84-88]. Additionally, no significant difference was observed in the rate of detection of *Helicobacter* species in intestinal biopsy specimens from 160 Chinese IBD patients (10%) and 80 controls (6.3%)[57]. Furthermore, in an earlier study assessing gastrointestinal mucosal lesions in children with IBD, infection with *H. pylori* was found in only 2 of 41 children with CD (4.8%) and in 5 of 47 with UC (10.6%)[89].

## *H. PYLORI* AND THE NATURAL HISTORY OF IBD

It is conceivable that *H. pylori* infection may influence the clinical course of CD by triggering both specific and nonspecific immune responses in the human intestine. Phenotype modification of CD was identified in a study in which seropositive non-smoking CD patients had significantly fewer relapses and a lower risk of bowel resection compared to seronegative non-smoking patients[90]. Moreover, serum anti-*H. pylori* IgG levels were significantly lower in subgroups of patients with fibro-stenotic and fistulising CD[54].

There are several hypotheses regarding how *H. pylori* may influence the host immune response and thus alter the clinical course of CD. *H. pylori* infection may exert a direct damaging effect via urease and cytotoxins on the ileal or colonic mucosa[91]. Moreover, *H. pylori* may induce an autoimmune-like reaction in the stomach with the production of anti-Lewis X and/or Y antibodies that have systemic auto reactive properties, thereby influencing the course of the disease[92]. Another mechanism could be the induction of platelet activation and aggregation as shown in murine gastric venules which can cause the formation of microthrombi in gastric and intestinal epithelium and lead to infarction and development of ulcers[93]. Another possibility is that *H. pylori* influences the host immune response via activation of the mucosa-associated lymphoid tissue (MALT), which may lead to a more generalized immune response to *H. pylori* infection in IBD, contributing to the initiation or perpetuation of inflammation. In fact, Duchmann *et al*[94] showed that bacteria-specific T cell clones are increased in inflamed intestinal mucosa of patients with IBD.

It appears that in *H. pylori* infected patients, CD is more often confined to the terminal ileum, a location that is frequently affected by complications, yet may be associated with a lower clinical disease activity[95]. *H. pylori* infection usually occurs early in life, before the onset of CD, so it is possible that this early infection may influence disease location in these patients[96]. As a result, *H. pylori* infection may not influence the course of the disease primarily, but may influences the location of the disease and thus secondarily alters its course.

## PROTECTIVE ROLE OF *H. PYLORI* AGAINST IBD

Many studies have reported that the prevalence of *H. pylori* infection is lower in patients with IBD compared to controls, demonstrating an inverse relationship between IBD and *H. pylori* infection that suggests a protective role of *H. pylori* infection against the development of IBD (Table 1)[32-34,38,39,41-46,48,49,52,55,57-59]. However this has not been confirmed by other studies (Table 1)[30,35-37,53,56,60]. Väre *et al*[41] found that seropositive CD patients presented at a significantly later age (40 years) compared to seronegative patients (30 years, $P < 0.001$), suggesting that the higher age of disease onset in seropositive IBD patients is the result of a protective modifier effect that *H. pylori* infection exerts on the development of IBD[41], although this has not been confirmed by other studies[34,42]. Furthermore, a meta-analysis of 23 studies suggested a protective role of *H. pylori* infection in CD pathogenesis, but the heterogeneity among enrolled studies and the possibility of publication bias limited the reliability of these results[97]. The published literature on the prevalence of *H. pylori* infection in UC and CD is diverse. Various studies have found a lower prevalence of this infection in CD compared to UC[29,31,34,41-43], whereas others have found exactly the opposite[34,35,55]; still others have reported no difference in the occurrence of *H. pylori* between the two diseases[30,32,33,36,37,39,48,56,57].

Moreover, the increased occurrence of *H. pylori*-negative FEG among IBD patients also confirmed the inverse association between the prevalence of *H. pylori* infection and IBD (Table 2). For example, *H. pylori*-negative chronic active gastritis was found in only 2% of patients without IBD compared to 20% of patients with IBD (CD 26%, UC 13%)[98]. Furthermore, permanent colonization of the stomach by *H. pylori* is unusual in children with IBD[40].

Heterogeneity among studies regarding the method of IBD and *H. pylori* diagnosis differences in study population, ethnicity and age across studies, and the possibility of publication bias may limit the certainty of the above findings. As environmental hygiene and intestinal

**Table 1 Prevalence of *Helicobacter pylori* infection in patients with inflammatory bowel disease in different populations**

| CD | UC | C | Control group | Method | Positive (%) | Country | Ref. |
|---|---|---|---|---|---|---|---|
| 42 | 51 | 40 | Patients with irritable bowel syndrome | UBT, *H. pylori* IgG (+) | IBD: 17.2, C: 25 CD: 11.9, UC: 21.6 | United Kingdom | [29] |
| 110 | 213 | 337 | Non-IBD patients with elective surgery[1] | *H. pylori* IgG (+) | IBD: 34.2, C: 36.2 CD: 33.3, UC: 34.7 | United Kingdom | [30] |
| 139 | 137 | 139 | patients with functional GI disorders[1] | *H. pylori* IgG (+) | IBD: 9.4, C: 16 CD: 5, UC: 14 | United Kingdom | [31][2] |
| 47 | 63 | 100 | Blood donors[1] | *H. pylori* IgG (+), UBT, histology | IBD: 21.8, C: 52 CD: 14.9, UC: 27, | United Kingdom | [32][2] |
| 67 | 41 | 43 | Non-IBD patients | Biopsies | IBD: 28.7, C: 39.5 CD: 28.4, UC: 29.3, | Italy | [33][2] |
| 123 | 93 | 216 | Blood donors[1] | *H. pylori* IgG (+), histology | IBD: 48.1, C: 58.8 CD: 40.7, UC: 55.9 | Italy | [34][2] |
| 32 | 40 | 72 | Healthy subjects[1] | UBT | IBD: 47.2, C: 61.1 CD: 53.1, UC: 42.5 | Italy | [35] |
| 12 | 8 | 29 | Patients with idiopathic constipation | UBT | IBD: 60, C: 41 CD: NR, UC: NR | Italy | [36] |
| 45 | 66 | 77 | Patients with non-organic dyspepsia[1] | histology | IBD: 66.7, C: 63.6 CD: 62.2, UC: 69.7 | Turkey | [37] |
| 0 | 90 | 120 | Healthy subjects | Histology, RUT | IBD: 30, C: 52.5 CD: NA, UC: 30 | Greece | [38][2] |
| 39 | 77 | 127 | Healthy subjects[1] | *H. pylori* IgG (+) | IBD: 31.7, C: 55.1 CD: 28.6, UC: 33.1 | Greece | [39][2] |
| 19 | 21 | NA | NA | *H. pylori* IgG, IgA (+), histology | IBD: 0, C: NA CD: NA, UC: NA | Finland | [40][3] |
| 94 | 185 | 70 | Healthy subjects[1] | *H. pylori* IgG, IgA (+) | IBD: 24.4, C: 37.1 CD: 12.9, UC: 29.7 | Finland | [41][2] |
| 100 | 100 | 100 | Patients with acute bacterial diarrhoea[1] | *H. pylori* IgG, IgA (+) | IBD: 15, C: 43 CD: 13, UC: 18 | Finland | [42][2] |
| 147 | 169 | 316 | Non-IBD patients[1] | UBT | IBD: 25.3, C: 52.5 CD: 17.7, UC: 32 | Korea | [43][2] |
| 386 | 0 | 277 | Blood donors[1] | *H. pylori* IgG, IgA (+) | IBD: 17.4, C: 35.4 CD: 17.4, UC: NA | Nederland | [44][2] |
| 90 | 0 | 525 | Non-IBD patients | Histology | IBD: 16.7, C: 40.2 CD: 16.7, UC: NA | Japan | [45][2] |
| 38 | 0 | 12 | Healthy subjects[1] | UBT | IBD:8, C: 42 CD: 8, UC: NA | Japan | [46][2] |
| 80 | 39 | 98 | Non-IBD patients[1] | *H. pylori* IgG (+) | IBD: 27.5, C: 41.7 CD: 13.5, UC: 30.8 | Israel | [47][2] |
| 51 | 82 | 200 | Non-IBD patients[1] | UBT | IBD: 12.8, C: 39 CD: 13.7, UC: 12.2 | Hungary | [48][2] |
| 36 | 0 | 36 | Healthy subjects[1] | Histology | IBD: 8.3, C: 36.1 CD: 8.3, UC: NA | Germany | [49][2] |
| 75 | 0 | 200 | Non-CD patients | Histology | IBD: 30.5, C: 35.2 CD: 33, UC: NA | Germany | [50] |
| 56 | 0 | 382 | Non-CD patients | Histology | IBD: 32.1, C: 46.1 CD: 32.1, UC: NA | USA | [51][3] |
| 371 | 560 | 64451 | Non-IBD patients | Histology | IBD: 4.5, C: 9 CD: 4, UC: 5 | USA | [52][2] |
| 0 | 42 | 74 | Non-IBD patients | *H. pylori* IgG (+), UBT | IBD: 52.4, C: 51.4 CD: NA, UC: 52.4 | Brazil | [53] |
| 43 | 0 | 74 | Non-IBD patients | UBT | IBD: 51.2, C: 70.3 CD: 51.2, UC: NA | Brazil | [54] |
| 50 | 44 | 194 | Non-IBD patients | Histology, RUT | IBD: 9.6, C: 38.5 CD: 14, UC: 4.5 | Poland | [55][2,3] |
| 21 | 23 | 76 | Non-IBD patients | *H. pylori* IgG (+) | IBD: 54.5, C: 68 CD: 52.2, UC: 57.1 | Mexico | [56] |
| 104 | 104 | 416 | Healthy subjects[1] | UBT | IBD: 19.7, C: 48.8 CD: 18.3, UC: 21.2 | Chinese | [57][2] |
| 229 | 0 | 248 | Non-CD patients | UBT, culture, histology | IBD: 27.1, C: 47.9 CD: 27.1, UC: NA | Chinese | [58][2] |
| 0 | 153 | 121 | Non-UC patients | UBT, culture, histology | IBD: 30.5, C: 57 CD: NA, UC: 30.5 | Chinese | [59] |
| 30 | 30 | 20 | Non-IBD patients[1] | UBT | IBD: 43, C: 40 CD: 50, UC: 37 | Spain | [60] |

[1]Age and sex matched; [2]Statistically significant result (IBD *vs* control group); [3]Paediatric population. CD: Crohn's disease; UC: Ulcerative colitis; C: Controls; IBD: Inflammatory bowel disease; GI: Gastrointestinal; *H. pylori*: *Helicobacter pylori*; Ref: References; NA: Not applicable; NR: Not reported; FAT: Serology fecal antigen test; RUT: Rapid urease test; UBT: Urea breath test.

**Table 2 Prevalence of both *Helicobacter pylori* negative and positive gastritis in patients with inflammatory bowel disease in different populations**

| CD | UC | C | Control group | Biopsies | *H. pylori* (+) gastritis (%) | *H. pylori* (-) gastritis (%) | Ref. |
|---|---|---|---|---|---|---|---|
| 37 | 43 | 41 | Non-IBD patients | Antrum, body | CD: 27, UC: 37.2<br>C: 53.7 | CD: 29.6, UC: 22.2<br>C: 10.5 | [61] |
| 141 | 79 | 141 | Non-IBD patients | Antrum, angulus, body | CD: 33, UC: 47<br>C: 60 | CD: 43, UC: 12<br>C: 19 | [34] |
| 75 | 0 | 200 | CD-free patients | Antrum, body | CD: 33.3, UC: NA<br>C: 48 | CD: 39, UC: NA<br>C: 0.8 | [50] |
| 208 | 280 | 4943 | Non-IBD patients | Antrum, body | CD: 4, UC: 6<br>C: 7 | CD: 5, UC: 0<br>C: 0 | [63] |
| 67 | 41 | 43 | Healthy subjects | Antrum, body | CD: 17.6, UC: 6.4<br>C: 20 | CD: 45.4 , UC: 15.6<br>C: 30 | [33] |
| 62 | 0 | 0 | NA | Antrum, corpus | CD: 9.7, UC: NA<br>C: NA | CD: 32 , UC: NA<br>C: NA | [64] |

CD: Crohn's disease; UC: Ulcerative colitis; C: Controls; IBD: Inflammatory bowel disease; *H. pylori*: *Helicobacter pylori*; NA: Not applicable.

microbiota may be strong confounders, further mechanistic studies in *H. pylori* infection using mouse models are necessary to further define the mechanism of this negative association. Furthermore, when looking for explanations for the lower prevalence of *H. pylori* infection in IBD, some authors have suggested that treatment with sulfasalazine and other aminosalicylic compounds could be responsible for "spontaneous eradication" of *H. pylori* infection[32,34,35,38]; although their possible role has not been confirmed by other studies[29-31,37,39,41-45,55,57,60,99]. Various studies have suggested that sulfasalazine, but not 5-aminosalicylic acid (5-ASA), could account for the lower prevalence of *H. pylori* infection[32,34], whereas Piodi *et al*[35] found the opposite. Ishikawa *et al*[100] observed a lower prevalence of *H. pylori* infection in rheumatoid arthritis patients receiving sulfasalazine, whereas Taha *et al*[101] did not find any statistically significant difference. The mechanisms of how these agents prevent *H. pylori* infection is still unknown, but prevention may be the result of a direct action against germ adhesion to the gastric mucosa or due to immuno-modulatory actions of the drugs[30,102,103]. It has also been hypothesized that prolonged treatment with antibiotics used in IBD (especially metronidazole) could account for spontaneous eradication and lower prevalence of *H. pylori* infection. Indeed the prevalence of *H. pylori* infection was significantly lower in CD patients who had received antibiotics for $\geqslant$ 2 wk[45] while in another study, antibiotic therapy was negatively associated with *H. pylori* infection (20.5% *vs* 55%, $P = 0.0001$)[39]. Moreover, other studies have shown that prior treatment with ciprofloxacin and/or metronidazole had no influence on *H. pylori* status in IBD patients[48,104,105].

Finally, the data on the prevalence of virulent *H. pylori* strains in IBD patients are limited. Wagtmans *et al*[44] showed that the majority (66%) of *H. pylori* seropositive patients with CD were infected by *H. pylori* cagA (+) strains although a similar proportion of controls (69.4%) were also infected by these strains. These findings deserve further investigation as it is well known that the intense host responses, specifically to *H. pylori* cagA (+) strains may further alter Th1- and Th2-type immune responses with subsequent induction of immune-regulatory lymphocytes[106].

## POTENTIAL PROTECTIVE MECHANISMS OF *H. PYLORI* AGAINST IBD

It is plausible to suggest that *H. pylori,* by attempting to promote its own survival, may benefit the host via a variety of mechanisms against other chronic inflammatory conditions such as IBD. Several mechanisms have been proposed to explain the inverse association between *H. pylori* and IBD. In CD, Th1 immune responses prevail, whereas in UC, Th2 or Th1/Th2 immune responses may be predominant[5,107,108]. These altered immune responses to lumen antigens in IBD may influence the way the host responds to *H. pylori* infection. Conversely, a perpetual bacterial infection in the stomach may either alter the host immune responses in a way that may be protective or render the host susceptible to IBD. The levels of numerous cytokines, including IFN-γ, TNF, IL-1β, IL-6, IL-7, IL-8, IL-10, and IL-18, are increased in the gastric epithelial cells of *H. pylori* infected humans compared to uninfected humans[109-111]. After activation of Toll-like receptors by *H. pylori,* dendritic cells (DC) may activate T cells in different ways, being capable of inducing either a Th1 or Th2/regulatory T cell (Treg) response by generation of IL-12 or IL-10, respectively[112,113]. This finding was reported by D'Elios *et al*[114] who observed that most (64%) of *H. pylori* specific T cell clones derived from uncomplicated chronic gastritis displayed a Th2-like phenotype, producing interleukin IL-4 or IL-5 together with INF-a, whereas only one third of *H. pylori*-specific gastric T cells were polarized with Th1 effectors.

Thus, a protective role of *H. pylori* infection against IBD may be due to the ability of this microbe to down-regulate pro-inflammatory immune responses. Considering that adoptive transfer of Treg is able to prevent and/or treat colitis in various animal models, it is reasonable to suggest that these cells produced in response to *H.*

*pylori* infection may act in the prevention of IBD[115-119]. *H. pylori* can induce a Treg response and down-regulate the pro-inflammatory Th1/Th17 pathway[65,66,120-123]. The importance of Treg in the pathogenesis of IBD was illustrated by the development of spontaneous colitis in mice deficient of IL-10, a key regulatory cytokine for Treg function[124].

Moreover, the systemic levels of type I IFN were found to be lower in *H. pylori* infection-colonized IBD patients compared to non-colonized controls[125]. Luther *et al*[125] showed that prior oral administration of 20-50 μg *H. pylori* DNA ameliorated the severity of dextran sulphate sodium (DSS) induced acute or chronic colitis in mice in terms of both pathology and symptoms such as bleeding and weight loss. Thus, the protective properties of *H. pylori* DNA were attributed *in vitro* to inhibition of cytokine production by DC, which upon addition of the DNA failed to produce type I interferon and IL-12 in response to *E. coli* DNA[125]. A protective effect of *H. pylori* colonization in mice against experimental colitis was also demonstrated by Higgins *et al*[126]. Mice that were colonized with *H. pylori* SS1 6 wk prior to the induction of *Salmonella typhimurium* experimental colitis, experienced markedly less severity inflammation compared to mice that were not colonized with *H. pylori*. This result could be attributed to an up-regulation of IL-10 in the mesenteric lymph nodes and suppression of the Th-17 response in the cecum of the infected mice[126], illustrating an extra-gastric immune-modulatory effect of the bacterium, an immunological crosstalk between the upper and lower gastrointestinal tract and providing mechanistic support for the epidemiological observation of a negative association between *H. pylori* status and the risk of IBD.

Another protective mechanism may operate via the development of antibodies against *H. pylori,* which may confer an immunization-type protection against other pathogenic *Helicobacter* or even different types of microbes implicated in IBD. Although *H. pylori*-specific antibodies do not eradicate this bacterium, they seem to confer a degree of protective immunity from a subsequent *Campylobacter* infection, indicating an antigenic cross-reactivity between these two bacterial species[127-129]. It could also be that *H. pylori* induced reduction in acid secretion indirectly affects a different type of infection that ultimately results in IBD. Indeed, variable disease phenotype during dual infection by different *Helicobacter* species has been described by Lemke *et al*[130] who demonstrated that *H. bilis* and *H. pylori* co-infection in mice attenuates *H. pylori* gastritis compared to those infected only with *H. pylori*.

The protective effect of *H. pylori* may simply be due to other confounding variables such as the presence of inherent genetic or environmental factors that favour *H. pylori* acquisition in some and the development of IBD in others. This scenario would fit well with the observation that IBD is associated with better hygiene, which in itself may be detrimental to *H. pylori* acquisition[131,132]. The low prevalence of *H. pylori* infection in patients with IBD compared to non-IBD patients strengthens the importance of the "hygiene hypothesis" in the development of autoimmunity and IBD. It suggests that inadequate microbial stimulation of gut-associated lymphoid tissue is a critical point for maturation of mucosal immunity[133,134]. Improved access to a cleaner environment and the resulting decreased incidence of common childhood infections, including *H. pylori,* may be contributing to autoimmunity by altering susceptibility to certain diseases with an autoimmune component, such as IBD[26].

Finally, regarding genetic factors, the CD variant of the autophagy gene ATG16L1 alters susceptibility to *H. pylori* infection with an enteric microbe in human subjects at the population level, supporting a role for altered autophagy in regulating the host response to enteric microbes in CD pathogenesis. It is interesting to speculate that due to increased susceptibility to infection, early exposure and acquisition of *H. pylori* in individuals with the ATG16L risk allele may decrease their risk for the subsequent development of IBD[135].

## ERADICATION OF *H. PYLORI* AND DEVELOPMENT OF IBD

There seems to be a rapid onset of CD after eradication of *H. pylori* infection, as illustrated by two cases[136]. A similar experience was recently described by Jovanovic *et al*[137], who described the onset of gastric CD only 6 mo after *H. pylori* infection eradication. Moreover, a steady rise in the incidence of UC was reported in *H. pylori* endemic regions after successful eradication of *H. pylori* infection[28].

It is unknown why these patients developed CD after eradication of *H. pylori* infection, but this may be due to the induction of immune responses that in turn contributed to the development of the disease. Long-term *H. pylori* infection may cause an unstable equilibrium between the Th1 and Th2 phenotype pattern; eradication of *H. pylori* infection may diminish Th2 cytokine, with sudden consequent Th1 pattern prevalence and rapid increase of pro-inflammatory cytokines[106]. In genetically predisposed subjects, this Th1 predominant pattern may suddenly favour the onset of a typical Th1-related disease such as CD. Further studies investigating the effect of eradication of *H. pylori* on the development and natural history of IBD are warranted.

## CAUSAL ASSOCIATION OF *H. PYLORI* WITH COLORECTAL CANCER?

A meta-analysis of 13 studies suggested an increased risk of colorectal cancer due to *H. pylori* infection[138]. Kapetanakis *et al*[139] demonstrated the presence of *H. pylori* in malignant colonic tissue in 34 of 41 (82.9%) patients with colorectal cancer. *H. pylori* colonizing colonic tumour tissue seems to be associated with increased cell proliferation and impaired apoptotic process in malignant tissue

compared with adjacent normal colonic mucosa, thereby further contributing to colon cancer progression[140]. Furthermore, *H. pylori* induced gastrin release can act as promoter of cell proliferation and differentiation (mainly by inducing COX-2 overexpression and PI3-kinase-mediated tyrosine phosphorylation of E-cadherin and b-catenin) in different gastrointestinal tract sites, including the colon[141]. *H. pylori* infection is also accompanied by bone-marrow–derived stem cell (CD34+) recruitment that ultimately facilitates colon cancer progression[142]. Finally, compared to normal gastric mucosa, *H. pylori* gastritis occurred more frequently among patients with hyperplastic polyps (OR = 1.24, 95%CI: 1.18-1.30), adenomatous polyps (OR = 1.52, 95%CI: 1.46-1.57), advanced adenomas (OR = 1.80, 95%CI: 1.69-1.92), villous adenomas or adenomas with high-grade dysplasia (OR = 1.97, 95%CI: 1.82-2.14), and adenocarcinomas (OR = 2.35, 95%CI: 1.98-2.80)[143]. It has therefore been proposed that *H. pylori* eradication might inhibit IBD-related or non-colon neoplasia[144].

## CONCLUSION

Since the discovery of *H. pylori*, several epidemiological studies, therapeutic trials, case reports and/or *in vitro* studies have been published concerning a hypothetical damaging or protective role of *H. pylori* in the development of IBD. Whether the link between *H. pylori* and IBD is coincidental, epiphenomenal or mechanistic remains uncertain. There are contradictory data regarding both the causative and the protective role of *H. pylori* infection against IBD.

The discordance between studies may be explained by a number of confounding factors, such as variability in the power of the studies and the time periods in which these studies were conducted, geographical factors and the differences in the methods used to detect *H. pylori* infection[145]. To be more specific, the urease breath test is more sensitive in detecting *H. pylori* than histology. Histology involves the examination of tissue samples that may be insufficient for a correct diagnosis and is more timely than serology, which also detects previous infections. Furthermore, one limitation of the studies using serology for the presence of *H. pylori* is the fact that after successful eradication of *H. pylori* infection, positive titres of antibodies normalize very slowly within several months, or even years, leading to the possibility that negative findings from *H. pylori* serology do not reflect eradication of *H. pylori* infection[146]. Finally, from a clinical point of view, we must always bear in mind that any type of protection that exerts its influence on a general population level may not necessarily materialize in the individual patient.

In conclusion, the association between *H. pylori* infection and IBD is still controversial; however, it is worthy of further investigation, as the potential association of *H. pylori* with extra-gastric manifestations and disorders is always a very interesting and challenging research area[147]. It is unclear whether the apparent protective effect of *H. pylori* is simply confounding due to other variables, but the effect of the presence of the live bacterium remains to be elucidated. More studies investigating the effect of *H. pylori* infection eradication on the risk of development of IBD and the natural history of IBD are needed.

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# Patients with established gastro-esophageal reflux disease might benefit from *Helicobacter pylori* eradication

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## Abstract

**Background** The aim of this study was to investigate the effect of *Helicobacter pylori* (*H. pylori*) eradication in selected *H. pylori*-positive patients with a primary diagnosis of gastro-esophageal reflux disease (GERD) by using the 3-h postprandial esophageal pH monitoring.

**Methods** We recruited patients with erosive esophagitis at endoscopy and *H. pylori* infection at histology, successfully cured following eradication therapy; the selected *H. pylori*-positive patients had weekly reflux symptoms for at least six months and endoscopically established Grade A or B esophagitis. Twenty-nine eligible patients were initially subjected to esophageal manometry and ambulatory 3-h postprandial esophageal pH monitoring. All patients received *H. pylori* triple eradication therapy accompanied by successful *H. pylori* eradication. After successful eradication of *H. pylori* (confirmed by $^{13}$C urea breath test), a second manometry and 3-h postprandial esophageal pH monitoring were introduced to assess the results of eradication therapy, after a 3-month post-treatment period.

**Results** All 29 selected *H. pylori*-positive patients became negative due to successful *H. pylori* eradication, evaluated by $^{13}$C urea breath test after a 4-week post-treatment period. Post-eradication, 62.1% patients showed similar manometric pattern at baseline; 17.2% showed improvement; 17.2% normalization; and 3.4% deterioration of the manometric patterns. The DeMeester symptom scoring in the 3-h postprandial ambulatory esophageal pH monitoring was improved after eradication of *H. pylori* (median 47.47 vs. 22.00, Wilcoxon's singed rank; P=0.016). On comparing the pH monitoring studies for each patient at baseline and post-eradication period, 82.8% patients showed improvement and 17.2% deterioration of the DeMeester score.

**Conclusion** By using 3-h postprandial esophageal pH monitoring, this study showed, for the first time, that *H. pylori* eradication may positively influence GERD symptoms. Large-scale controlled relative studies are warranted to confirm these findings.

**Keywords** *Helicobacter pylori*, gastro-esophageal reflux disease, esophageal pH monitoring, DeMeester symptom scoring

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## Introduction

*Helicobacter pylori* (*H. pylori*) infection is a well-known etiological factor for many gastrointestinal diseases, such as chronic gastritis, peptic ulcer disease, mucosa-associated lymphoid tissue lymphoma, and gastric cancer. The gastric inflammation due to *H. pylori* may be antral-predominant gastritis, closely associated with duodenal ulceration, whereas corpus-predominant gastritis is associated with an increased risk of gastric cancer, though *H. pylori* atrophic gastritis affects both antral or corpus mucosa (multifocal atrophic gastritis). Eradication of *H. pylori* infection is recommended to prevent and/or treat these diseases. In addition, there are many important issues to be elucidated regarding the role of *H. pylori* in very severe pathologies such as esophageal cancer and other more benign disorders, common in the developed world, such as gastro-esophageal reflux disease (GERD), which carry a significant impact on health economics, and

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Conflict of Interest: None

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patient morbidity. In this respect, symptoms like heartburn, acid regurgitation, and dysphagia are usually sufficient to confirm the diagnosis of GERD and initiate treatment. The most common test used to confirm excessive GERD is ambulatory 24-h esophageal pH monitoring; although this test cannot be regarded as a definitive gold standard for GERD diagnosis, it is indicated in several clinical situations defined by national or expert groups. The main limitation of the 24-h pH monitoring is its low tolerability [1]; patients report that pH testing frequently induces unpleasant side effects lasting for most of the day. Thus, a shorter monitoring period may be more tolerable [2].

To our knowledge, there are no data regarding the evaluation of the effect of *H. pylori* eradication in GERD patients, by using the 3-h postprandial esophageal pH monitoring. Therefore, the aim of this study was to evaluate the effect of *H. pylori* eradication in a Greek cohort with *H. pylori*-positive GERD, by using the 3-h postprandial esophageal pH monitoring, as a more flexible test for evaluating this disease.

## Patients and methods

### Patients

Due to protocol, by introducing mainly the 3-h postprandial esophageal pH monitoring, we enrolled patients who had erosive esophagitis at endoscopy and *H. pylori* infection at histology, successfully cured following eradication therapy. Specifically, the selected *H. pylori*-positive patients had weekly reflux symptoms for at least six months. The twenty-nine selected *H. pylori*-positive patients who were eligible underwent esophageal manometry and ambulatory 3-h postprandial esophageal pH monitoring at baseline and 3-months post-*H. pylori* eradication regimen; successful eradication of *H. pylori* was observed in all 29 selected *H. pylori*-positive patients, confirmed by $^{13}$C urea breath test (UBT) at 4-week post-treatment period. All participants provided informed consent. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the local ethics committee.

Exclusion criteria for the GERD patients were: any past history of gastric or esophageal surgery; suspected or confirmed malignant disease; previous *H. pylori* eradication regimens; anticoagulant treatment; esophageal ring stricture or esophagitis secondary to systemic diseases (e.g. scleroderma or ingested irritants); primary esophageal motility disorders; pregnancy or lactation; and age <18 years old. Patients with endoscopic evidence of active gastrointestinal bleeding and those with Zollinger-Ellison's syndrome were also excluded from the study. All patients had stopped acid suppression therapy (4 days beforehand for those taking antacids and 20 days beforehand for those using $H_2$-receptor antagonists or proton pump inhibitors) and underwent a 4-week washout period during which any medications known to affect gastrointestinal motility, like tricyclic antidepressants, were tapered. None of the patients was receiving oral medication that could cause or deteriorate GERD symptoms [3].

### Endoscopy

All 29 selected patients were seen at 9 a.m. after a 12-h fast. Intravenous sedation was given, and standard upper gastrointestinal endoscopy, using the Fujinon EPX-201 endoscopy system (Fujinon Optical Tokyo, Japan), was performed to identify evidence of macroscopic abnormalities. The degree of reflux esophagitis was graded from A (least severe) to D (most severe) according to the Los Angeles classification system. Two biopsy specimens were obtained from the antral region within 2 cm of the pyloric ring from each patient. One biopsy specimen was used for rapid urease slide testing of *H. pylori* infection (CLOtest) and the other biopsy specimen was placed in 10% formalin and submitted for histological examination to look for *H. pylori* organisms on Giemsa staining; the diagnosis of *H. pylori* infection was confirmed by histology.

### Manometry and 3-h postprandial pH-monitoring study

All 29 patients were reviewed for baseline manometry and 3-h postprandial pH monitoring within 3 days. After an overnight fast esophageal manometry was performed using a 4-channel, silicone rubber, low compliance, pneumohydraulic-perfused manometric assembly without a sleeve sensor (Manometric pump-model PIP-4-8SS Mui Scientific). The manometric assembly was passed transnasally and the position of the lower esophageal sphincter (LES) was determined using the station pull-through technique at 0.5 cm intervals. Manometric examination of the LES also served as a guide for the correct placement of the pH-sensitive probe. After removal of the manometric catheter, a monocrystalline antimony pH catheter was passed transnasally and the electrode was positioned 5 cm above the proximal margin of the LES. The electrode was calibrated in buffers of pH 7 and pH 4 before each study (pHmetry UPS 2020, MMS- Medical Measurement Systems BV). The 3-h postprandial ambulatory esophageal pH monitoring study was then carried out. Patients were encouraged to avoid coffee, alcohol, fruit juices, and antacids. They were also encouraged to take their meal at the same time during the 3-h period. They were however instructed to proceed with their normal daily routine. The same meals were used during the second pH recording to minimize day-to-day pH variation. Data acquisition was performed using a portable solid-state data logger. The pH data were analyzed by a standard software programme. The start of a reflux episode was defined by an esophageal pH below the threshold of 4.0 and its end by an esophageal pH above 4.0. Parameters of esophageal acid exposure, including the DeMeester score, were then calculated for the 3-h postprandial period by standard software programme. All analyses were performed by a single investigator (J.M.). Abnormal GERD, or a positive test, was defined as pH<4 in the distal esophagus

for more than 4% of the total recording time and DeMeester score >14.72 [4]. The DeMeester score, used as the basis for correlation between the subjects, was calculated for the 3-h postprandial period by a standard software program. The DeMeester score is a complex index that takes into account the percentage of total time with pH<4, pH<4 in upright position, pH<4 in supine position, the number of reflux episodes with intra-esophageal pH<4, the number of reflux episodes with intra-esophageal pH<4 with duration over 5 min and the reflux episode with the greatest duration in min.

### *H. pylori* eradication regimen

All 29 *H. pylori* infected patients received the 10-day regimen including use of rabeprazole 20 mg, amoxicillin 1 g, and clarithromycin 500 mg b.i.d. for 10 days, followed by rabeprazole 20 mg q.d. for another 30 days. Subsequently, the $^{13}C$ UBT was used to confirm *H. pylori* eradication at 4-week post-treatment period, thereby avoiding false negative UBT results if rabeprazole was not suspended 4 weeks before performing the test. Manometry and 3-h postprandial pH-monitoring studies were also repeated at a 3-month post-treatment period (~20 days post-UBT period) to define any changes in these parameters, following *H. pylori* eradication.

### Statistical analysis

Statistical analysis was performed using the SPSS for Windows package (version 11.0, SPSS, Chicago, IL, USA). Data were presented as means ± SD, means with 95% CI or median values with 5$^{th}$ and 95$^{th}$ percentiles when appropriate. Wilcoxon's rank test was used to detect differences between baseline and 3-month *H. pylori* post-eradication period. For categorical variables, differences in frequencies were studied using the Fisher's exact test. Significance was set at P<0.05.

## Results

All 29 selected patients had endoscopically established Grade A or B esophagitis according to the Los Angeles classification system. Moreover, according to protocol, all 29 patients received successful *H. pylori* eradication therapy, as confirmed by UBT at 4-week post-treatment period.

Post-eradication, 18 out of 29 *H. pylori* positive patients (62.1%) showed the same manometric pattern as baseline; 5 patients (17.2%) showed improvement (propagation of esophageal peristalsis, pressure of LES); 5 patients (17.2%) normalization; and 1 patient (3.4%) deterioration of the manometric patterns (Table 1).

The overall DeMeester score in the 3-h postprandial ambulatory esophageal pH monitoring improved after eradication of *H. pylori* (median 47.47 vs. 22.00, Wilcoxon's singed rank; P=0.016) (Fig. 1). Specifically, on comparing the pH monitoring studies for each patient prior and after successful *H. pylori* eradication, 24 of 29 patients (82.8%) showed improvement; and 5 of 29 the patients (17.2%) showed deterioration of the DeMeester score. Moreover, 9 of the 24 (37.5%) patients with improved pH monitoring study, during the post-eradication period showed completely normal pHmetry (DeMeester score <14.72) post-treatment.

**Table 1** Post-eradication manometric pattern in *Helicobacter pylori*-positive patients with gastro-esophageal reflux disease (GERD)

| Manometric pattern | GERD patients n=29 | % |
|---|---|---|
| Normalization | 5 | 17.2 |
| Improvement | 5 | 17.2 |
| Stable | 18 | 62.1 |
| Deterioration | 1 | 3.4 |

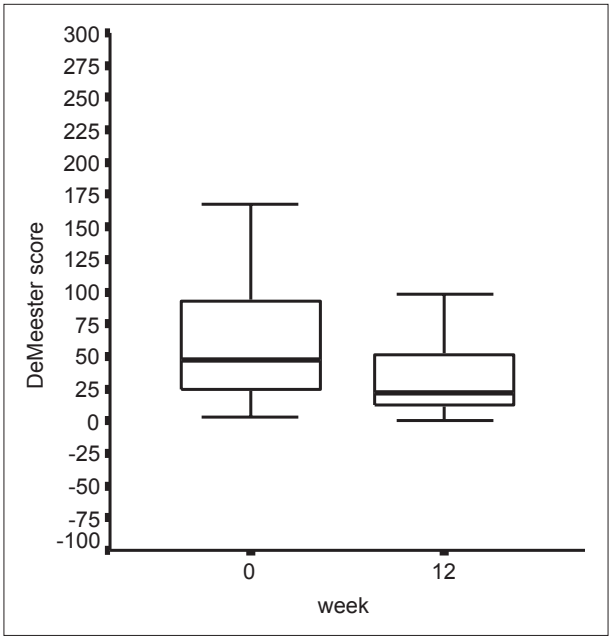


**Figure 1** DeMeester score at baseline and 4-week post-eradication period in 29 *Helicobacter pylori*-infected patients with gastro-esophageal reflux disease. Bold horizontal lines express median values, boxes represent interquartile range

## Discussion

To our knowledge, this is the first study aiming to evaluate the effect of *H. pylori* eradication in a cohort with GERD, by using 3-h postprandial esophageal pH monitoring; three months after successful eradication of *H. pylori* there was significant improvement in the severity of acid reflux, as judged by established criteria. Although analysis of esophageal and stomach acidity is the best method of studying the pathogenesis of GERD, it is difficult to perform 24-h pH monitoring in a

large number of patients, due to poor compliance. We therefore used the 3-h postprandial pH monitoring study as a sensitive and mainly more flexible method; the overall DeMeester score in the 3-h postprandial ambulatory esophageal pH monitoring was significantly improved with a concomitant improvement in pH monitoring after eradication of *H. pylori* in our patients.

To our knowledge, four relative studies exist in the literature comparing GERD before and after *H. pylori* eradication by using 24-h pH monitoring.

Verma *et al* [5], aimed to assess the prevalence of GERD before and after *H. pylori* eradication by using 24-h esophageal pH/manometry studies. Patients were followed up at 6 months and 1 year when they underwent a repeat 24-h pH/manometry; 20 patients were enrolled, though only 10 patients attended for a repeat 24-h pH/manometry study. *H. pylori* eradication: a) had no impact on percentage of time pH <4 and DeMeester Score; and b) induced no substantial changes in LES pressure and other esophageal manometry data. It is important to note that new onset GERD occurred very unusually one year after *H. pylori* eradication [5].

Tefera *et al* [6], expected that *H. pylori* eradication might increase GERD in reflux esophagitis patients, because increased prevalence of esophagitis has been reported following eradication of *H. pylori*. Twenty-five consecutive patients with *H. pylori* infection were enrolled; 24-h intra-esophageal pH recording was performed before and 12 weeks after eradication. *H. pylori* eradication, also confirmed by $^{13}$C UBT, induced no consistent change in gastro-esophageal acid reflux.

Manifold *et al* [7] studied 25 patients with *H. pylori* gastritis using 24-h esophageal and gastric pHmetry and gastric bilirubin monitoring before and after *H. pylori* eradication, also confirmed by $^{13}$C UBT. No differences were noticed in esophageal acid reflux, gastric alkaline exposure, or gastric bilirubin exposure before and after eradication. The authors concluded that *H. pylori* eradication induces no change in GERD or duodenogastric reflux.

Wu *et al* [8] also studied 25 patients with *H. pylori* erosive esophagitis using 24-h esophageal pHmetry. They concluded that *H. pylori* eradication increases esophageal acid exposure and might adversely affect the clinical course of disease in 21% of patients.

Differences in populations enrolled and methodologies might explain, at least partly, the discrepancies of our own and Verma's, Tefera's, Manifold's, and Wu's findings. Nevertheless, our and Verma's data indicate that *H. pylori* eradication might protect against GERD development [9].

In this regard, *H. pylori* infection is frequent in Greek patients with GERD and even with non-endoscopic reflux disease and *H. pylori* eradication leads to better control of GERD symptoms and improves esophagitis [10]. Moreover, consistent associations with the Greek data were shown by others [11], also reporting improvement in reflux symptoms following *H. pylori* treatment. It is important to note that some other authors, usually prior supporters of the theory that *H. pylori* "protects" against GERD, relented their initial findings, claiming that *H. pylori* eradication does not cause or protect against GERD, and, moreover, recommending *H. pylori* eradication in GERD [12]. Additionally, although epidemiologic studies do not suggest causality with *H. pylori*, however, such studies support our and others' findings; for instance, a large study (~21,000 cases) showed that the decrease in *H. pylori* infection parallels the decrease in peptic ulcer prevalence, and the increase in GERD and reappearance of GERD after *H. pylori* eradication is rare [8], also reported by Verma *et al* [5]. Much evidence further potentiates the concern that *H. pylori* is not "protective" against GERD [13].

The interplay between *H. pylori* and host factors plays an important role in the pathogenesis of GERD. Specifically, *H. pylori* may contribute to GERD pathogenesis by several mechanisms including the release of several mediators, cytokines and nitric oxide (NO) which may adversely affect the LES; cause direct damage of the esophageal mucosa by bacterial products; increase production of prostaglandins that sensitize afferent nerves and reduce LES pressure; and augment acidity (by gastrin release) that exacerbates GERD [10,14]. Specifically, there is the concept that gastric inflammation at the cardia may lower the threshold for transient relaxationof the LES by altering the sensitivity of vagal sensory receptors [15]. *H. pylori* gastritis is accompanied by release of the abovementioned NO, cytokines and prostaglandins that promote damage to the adjacent esophageal mucosa [16]. There is good evidence to indicate that the excessive production of prostaglandins in reflux esophagitis drives a vicious cycle of LES dysfunction, reflux, mucosal inflammation, aggravated LES dysfunction and further reflux. Moreover, the predominantly antral *H. pylori* gastritis is associated with an augmented gastrin release; increased acidity along with a higher volumeof gastric juice may aggravate reflux disease. Finally, in a recent critical review [17] regarding the complicated data available on the topic of *H. pylori* association with GERD, the authors concluded that: Intra-esophageal pH recording data fail to confirm increased acid reflux following *H. pylori* eradication; esophageal manometric studies suggest that bacterial eradication reduce rather than favor acid reflux into the esophagus; clinical studies suggest that *H. pylori* eradication is not considerably associated with reflux symptoms or erosive esophagitis onset; and some data also suggest an advantage in curing the infection when esophagitis is already present.

The present series have certain limitations: 1) the sample size, though the biggest in the relevant literature, was rather small, given that even the introduction the 3-h postprandial esophageal pH monitoring is not always easily acceptable by the patients; 2) the missed patients with a persistent *H. pylori* infection may preclude assessing whether the observed pH-recording modifications are definitely due to the bacterial loss; and 3) only 1 biopsy specimen was used for histology. This is an inadequate sampling protocol for routine endoscopic practice; 5 biopsies (2 antrum, 1 angulus, 2 corpus) being advised by current guidelines for an accurate upper endoscopy. However, due to large number of patient recruitment to find our selected *H. pylori* positive GERD patients, we were obliged to take only 1 instead of 5 biopsies for many obvious reasons mainly including the time burden of histological evaluation.

In conclusion, this study shows that *H. pylori* eradication may positively influence GERD symptoms. However,

**Summary Box**

**What is already known:**

- The most common test used to confirm gastro-esophageal reflux disease (GERD) is ambulatory 24-h esophageal pH monitoring, though characterized by a low tolerability
- Intra-24-h esophageal pH recording data rather fail to confirm increased acid reflux following *Helicobacter pylori* (*H. pylori*) eradication

**What the new findings are:**

- By using the 3-h postprandial esophageal pH monitoring as a more flexible method, this study showed, for the first time, that *H. pylori* eradication may positively influence GERD symptoms

large-scale controlled relative studies are warranted to evaluate these findings in depth.

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# "Concomitant" or "sequential" eradication of *Helicobacter pylori*: which regimen comes first?

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**Title:** Randomised clinical trial comparing sequential and concomitant therapies for *Helicobacter pylori* eradication in routine clinical practice

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## Summary

"Sequential" or "concomitant" non-bismuth quadruple regimens are currently recommended by the recent updated (Maastricht IV) European guidelines as alternative to bismuth based quadruple regimen first line therapies, in areas with a high rate (over 20%) of clarithromycin resistance [1]. Besides this, there is no trial comparing both regimens in settings with increasing rates of clarithromycin resistance [2]. In a recent prospective, randomized, multicenter, clinical trial, conducted in Spain, McNicholl *et al* aimed to compare the effectiveness and safety of these therapies for *Helicobacter pylori* (*H. pylori*) eradication [3].

They included a large number of patients (n=338) with non-investigated/functional dyspepsia (80%) or peptic ulcer disease (20%), naïve to eradication therapy. Mean age was 47 years, 60% were women and 20% smokers. They were randomly assigned to sequential treatment; omeprazole (20 mg/12 h) and amoxycilline (1 g/12 h) for 5 days, followed by 5 days of omeprazole (20 mg/12 h), clarithromycin (500 mg/12 h) and metronidazole (500 mg/12 h)[170 patients (50.3%)] or concomitant treatment; same drugs at the same doses taken concomitantly for 10 days [168 patients (49.7%)]. Eradication was confirmed with $^{13}$C-urea breath test or histology (depending on the indication), at least 4 weeks after treatment. Treatment related adverse events and adherence to treatment were also carefully evaluated.

A total of 302 patients completed the follow up and were tested for *H. pylori* eradication. The success rate of either regimen was defined as the primary outcome measure and was expressed both by intention-to-treat and per protocol. Secondary outcomes included the rate of treatment-emergent adverse events (AE's) and patients' adherence to treatment. Concomitant and sequential eradication rates were respectively, 87% versus 81% by intention-to-treat (P=0.15) and 91% versus 86% (P=0.13%) per protocol. Multivariate analysis showed an odds ratio of 1.5 towards better eradication rate with concomitant regimen of borderline significance (OR 1.5, 95% CI 0.9-2.8). Respective adherences to treatment were satisfactory and comparable between treatments (83% versus 82%). AE's were reported by as many as 59% of their patients but were mostly mild (60%), leading to treatment discontinuation in only 12 patients. In conclusion, the concomitant regimen had a non-significant advantage over sequential therapy and was the only one overcoming the 90% cure rate, per protocol. Both therapies were well tolerated and safe.

## Opinion

*H. pylori* is a global human pathogen that plays an essential role in the pathogenesis of prevalent diseases, including peptic ulcer disease and gastric malignancy [4]. Therefore, this infection should be cured whenever it is diagnosed [5]. Due to globally increasing prevalence of clarithromycin resistance [6,7], clarithromycin-based standard triple therapies have lost their efficacy [8] and should be abandoned as first line therapies, in several parts of the world, including most European countries and Greece [9,10].

Accordingly, the current European guidelines preclude the use of empiric standard triple therapies in areas with high prevalence of clarithromycin resistance (over 20%) and instead they recommend as first-line treatments, bismuth-based or alternatively non-bismuth quadruple regimens (the so called "concomitant" or "sequential" regimens). As bismuth salts are currently unavailable in several countries the usage of non-bismuth quadruple therapies is becoming inevitable [11]. Up to now, there are only a few head-to-head studies comparing sequential and concomitant regimens but either they took place in low clarithromycin resistance settings [12,13] or the comparison of the two regimens was unfair (i.e. 5 days concomitant versus 10 days sequential) [14]. The trial conducted by McNicholl *et al* is the first one randomly comparing concomitant and sequential regimens, both of 10 day duration, in a European setting with increasing rates of clarithromycin

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Conflict of Interest: None

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resistance. This is a well-designed study, conducted in a clinical practice setting, thus representing the "real-world" situation (i.e. the effectiveness of treatment regimes in the usual, every day practice). Unfortunately, in routine clinical practice, antibiotic susceptibility testing is neither usually performed nor indicated and this was the major limitation of this study, as acknowledged by the same authors [3]. The absence of culture based estimates limits generalization of study results and their reproducibility in areas exhibiting different patterns of antibiotic resistances [15]. In addition, if we extrapolate the results of the recent European survey on antibiotic resistance concerning Spain (clarithromycin resistance 14% and metronidazole 28%) to the study population, then we refer to a population with moderate and not high (over 20%) rate of clarithromycin resistance [16]. There are many available data in a number of recent studies, showing that bacterial resistance to key antibiotics (clarithromycin and/or metronidazole) adversely affects treatment outcome either with sequential or concomitant therapy [15,17-19]. Up to now, there is no non-bismuth quadruple therapy which totally overcomes bacterial resistance [20] but it is also true that concomitant regimen seems to work better than sequential against dual and possibly metronidazole resistant strains of *H. pylori,* as we have efficiently shown in an ongoing, randomized, multicenter trial [21]. In contrast, a recent meta-analysis [22] has shown equal efficacy of both regimens but it has been criticized for the inclusion of two studies that unfairly compared concomitant and sequential regimens of different duration [23,14,24]. When both regimens are fairly compared, concomitant shows a significant advantage over sequential [21,23].

The small difference (delta) in eradication success rates among these regimens, recorded in different studies, is expected to get wider and statistically significant as we move from low (i.e. Taiwan, 7%) to moderate (i.e. Spain, 14%) and high (Greece, 26%) clarithromycin resistance areas (delta value = 0%, 5% and 10%, respectively) [12,3,21]. This fact probably reflects the divergent effects of these regimens on antibiotic resistant strains and should properly be addressed in future trials. As there is no size that fits all, the answer to the question: "Which regimen comes first in *H. pylori* eradication?" is not straightforward and largely depends on the level of bacterial resistances in a defined population [25].

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