

Helicobacter pylori infection: A recent approach to diagnosis and management

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Abstract

Helicobacter pylori infection is the most common infection worldwide and is associated with simple dyspepsia, heartburn and peptic ulcer diseases, most commonly leading to upper gastrointestinal bleeding and, ultimately, to the severe complication of gastric malignancy. Ninety percent of duodenal ulcers and 70% of gastric ulcers are associated with helicobacter pylori infections. Noninvasive methods, such as the urea breath test (UBT-13C or 14C) and fecal antigen test (FAT), have high sensitivity and specificity for the diagnosis of H. pylori. However, 4-6 weeks of anti-secretory drugs (proton pump inhibitor) prior to testing can lead to false negative results. Invasive methods have benefits over noninvasive methods in the case of peptic ulcer diseases by taking samples for culture to determine their sensitivities and to stage disease progression to malignant transformation. Initially, standard triple regimen was the choice for treatment worldwide. However, high antibiotic resistances in various geographical regions have recently made standard quadruple therapy (bismuth-based) the preferred treatment. Recent studies have shown the promising benefits of 10-14 days of sequential and concomitant quadruple therapies as the first line option in high drug resistance areas, in cases of multidrug resistance or in prior treatment failure cases. Levofloxacin-based triple therapy, furazolidone-based regimens, and recent hybrid or rescue therapies are also beneficial towards the eradication of H. pylori infection as a second or third line therapy. Additionally, the use of probiotics and phytomedicines improve eradication rates when used with triple or quadruple therapies.

Key words: Helicobacter pylori, Diagnosis, Treatment, Antibiotic resistance

Introduction

Helicobacter pylori infection is one of the primary causes of upper gastrointestinal diseases, including dyspepsia, peptic ulcer diseases, heartburn, gastroesophageal reflux disease and even malignant transformation. It is the most common infectious human pathogen, infecting more than 50% of the populations worldwide (approximately 30% of children and 60% of adults), and is associated with 70% of benign gastric ulcers and 90% of duodenal ulcers [1, 2]. The relative risk of gastric cancer in a HP

positive group is 1.7-5.3 times higher than that in a HP negative group [3, 4]. It is more prevalent in developing nations compared to developed nations, indicating that socioeconomic status and living standards may play a major role in the distribution of infection. However, recent data showed a declining prevalence of HP infection in adults (individuals aged <40 years) [4, 5]. The global prevalence of HP infection, according to regions, is the following: 7-30% in North America, 30-90% in South America (mostly

in Chile and Brazil), 1.2-70% in Europe (least in the Netherlands, 1.2%), 13-90% in Asia (mostly in Bangladesh and India), 50-94% in the Middle East (mostly in Albania, Bulgaria, and Estonia), 15-20% in Australia, and 48-95% in Africa (mostly in Ethiopia) [6]. In China, it is more prevalent in those aged >60 years (86%) and, surprisingly, is more prevalent in vegetarians [7]. The annual HP re-infection rates in the West are reported to be 0.5-2.5% compared to 4.3%-13% in Asia [4].

As the eradication of HP infection plays a prime role in reducing the potential risk of complications, such as peptic ulcer disease, MALT lymphoma, and gastric carcinoma, HP eradication therapy performed before 30 years of age has nearly a 100% chance of preventing gastric cancer. However, the chance of preventing gastric cancer decreases to 41% in men and 71% in women when eradication is performed after 70 years of age [8, 9]. Therefore, the aim of this review is to discuss the recent and appropriate diagnosis and management that are available for *H. pylori* infections for reducing the lifetime risk of gastric cancers.

Risk factors

As the volume of drug distribution is higher in obese patients with a high BMI, the risk of drug failure is higher, most likely due to the reduced concentration of drugs at the gastric mucosal level. By contrast, lower basal metabolic index (BMI) patients (especially Asian) will have better outcomes [10]. Smoking is another risk factor for failure of therapy. A meta-analysis showed that the summary odds ratio for eradication failure in smokers versus non-smokers was 1.95 (95% CI 1.55-2.45), with a corresponding mean difference of 8.4% in eradication rates. The reason for this finding may be due to reduced gastric blood flow or intragastric pH in cases of smoking or nicotine, which could potentiate the vacuolating toxin activity of *H. pylori* in gastric cells [11, 12].

Pathogenesis

Helicobacter pylori, a Gram-negative, helical, rod-shaped bacterium is commonly indwelling in the luminal surface of the gastric epithelium. Typical transmission is via feco-oral or oro-oral routes. Not all infected individuals with *H. pylori* develop clinical diseases; the reasons for this variation may be bacterial pathogenicity and host susceptibility. Ninety to ninety-five percent of patients with duodenal ulcers and 70% of those with gastric ulcers are associated with *H. pylori* infections. Therefore, it is the main cause of peptic ulcer diseases associated with or without upper gastrointestinal bleeding.

Additionally, it is a risk factor for mucosa-associated lymphoid tissue (MALT) lymphoma and gastric adenocarcinoma (mostly noncardia) [13]. Recently, systematic reviews and a meta-analysis revealed that extra gastric diseases are also associated with *H. pylori* infections, such as unexplained iron deficiency anemia, idiopathic thrombocytopenic purpura, unexplained vitamin B12 deficiency, and the association of CagA-positive HP strains with ischemic heart disease [14]. The pathogenesis of *H. pylori* includes the following: (i) bacterial colonization in the gastric epithelium (invasion of gastric epithelium); (ii) bacterial virulence factors, including cytotoxin associated gene A (CagA), CagL and type IV secretion system (T4SS), vacuolating cytotoxin A (VacA), and Cag pathogenicity island (PAI) strains (associated with higher inflammatory response); (iii) environmental regulations of virulence factors; (iv) genetic diversity related to virulence; (v) host factors implicated in pathogenesis, including VacA and autophagy, oxidation and antioxidation response, apoptosis, and epigenetic changes, genetics or immune response [15-18]; and (vi) *H. pylori* colonization in the oral cavity, which significantly correlates with gastroesophageal diseases and could cause re-infection and attenuate the eradication rates of the disease [19].

Diagnosis

Patients with HP infection may clinically present with dyspepsia, heartburn, abdominal pain, diarrhea, or halitosis. A specific diagnosis can be made by available invasive and non-invasive methods (Table 1). Both methods have good sensitivity and specificity; however, their applicability may vary from country to country.

Invasive methods

Gastroduodenoscopy is very essential in symptomatic individuals who are not responding to therapy and those aged older than 45 years according to European guidelines [20]. It is not only diagnostic but also therapeutic in upper gastrointestinal bleeding (UGIB) cases. Additionally, it is a very important procedure in taking biopsies to diagnose *H. pylori* infection using RUT, a histopathological evaluation, culture and sensitivity. These invasive tests have good sensitivity and specificity; however, the chance of false negative results is the drawback that decreases the diagnostic accuracy of this method due to an uneven distribution of *H. pylori* in the gastric mucosa of the biopsied site and numbers of sampling [4].

Table 1. Various diagnostic methods for diagnosis of *H. pylori* infection

Tests	Sensitivity	Specificity	Diagnosis		Advantages	Limitation
			Pre-treatment	Post-treatment		
Non-invasive method:						
13 C-UBT[21, 22, 45, 46]	95-100%	90-100%	Yes	Yes	Widely available, reliable, simple, rapid	Special equipment required
14C-UBT	92% PPV-100% NPV-84%	100% Accuracy 94%	Yes	Yes		May have risk in pregnancy
Monoclonal stool antigen test (SAT) [21, 22]	90-95%	90-95%	Yes	Yes	Simple, reliable, rapid	Low compliance
IgG serology 8-14	85%	80%	Yes	No	Widely available, simple	Positive in post eradication
Invasive method:						
Rapid urease test [21, 22]	90-95%	91-100%	Yes	Yes	Simple, rapid	High bacterial load
Histology [21, 22, 27]	90-100%	93-99%	Yes	Yes	Sensitive, secondary diagnostic	Time consuming
Immuno-histochemistry			Yes	Yes	Excellent sensitivity	Expansive, limited use
Culture and sensitivity [31]	96%(MCM) 54%(CC)	80%(MCM) 100%(CC)	No	Yes	Show antibiotic sensitivity	Limited availability, low compliance
PCR [38]	97%	91%	Yes	Yes	Quick, antibiotic sensitivity	Expansive, limited availability

13/14 C-UBT- 13/14 carbon urease breath test, SAT- stool antigen test, PPV- positive predictive value, NPV- negative predictive value, IgG- immunoglobulin G, MCM- microcapillary culture method, CC- classical culture, PCR- polymerase chain reaction

Rapid urease test (RUT)

RUT is a biopsy based on a characteristic urease reaction. It is simple and rapid, with very good sensitivity and specificity, even in post-treatment eradication cases. The sensitivity and specificity varies from 90-95% and 91-100%, respectively [21, 22]. However, the number of bacteria present in the biopsy sample mainly affects the sensitivity of the test [23], and non-helicobacter urease producing organisms (*Proteus mirabilis*, *Citrobacter freundii*, *Klebsiella pneumonia*, *Staphylococcus aureus*, and *Enterobacter cloacae*) may cause false-positive results in patients with achlorhydria. Therefore, positive test results after 24 hours should be discarded to rule out false positive test findings [24, 25]. The accuracy of RUT is low, especially in the presence of a gastric hemorrhage compared to SAT. However, gastroduodenoscopy is diagnostic and therapeutic in upper gastrointestinal bleeding, although RUT may not be accurate in the diagnosis of HP-associated upper GI bleeding [4].

Histology

Histology remains the gold standard for diagnosis of HP gastritis and detection of *H. pylori* organisms, with a sensitivity and specificity >95% [6, 21]. Giemsa staining is a widely used technique, and immunostaining would increase the sensitivity and specificity to 100% and 98-99%, respectively [5, 12, 26-28]. Furthermore, it is very informative regarding the degree of inflammation and even for categorizing post helicobacter infective gastric mucosal changes (atrophic gastritis, intestinal metaplasia, mucosa associated lymphoid tissue (MALT) lymphoma,

malignancy) [28]. A biopsy sample from the body of the stomach is preferred compared to one from the antrum in patients taking acid suppressing drugs, or it can be performed after 2 weeks after drug discontinuation [5, 20]. The availability of immunohistochemistry is limited; however, it has advantages in diagnosing HP infection in RUT negative cases and HP CagA genotypes [29, 30].

Culture and sensitivity

The role of culture and sensitivity plays a prime role in increasing *H. pylori* eradication rates as it not only diagnoses *H. pylori* infection but also guides clinicians on using the appropriate treatment regimen according to the sensitivities of drugs in particular geographical regions where antibiotic resistance is high. The classical culture method has a lower sensitivity of 65% compared to the newer microcapillary cultivation method, which has a sensitivity and specificity of 96% and 80%, respectively [31]. This test is not a common choice in areas where there are lower drug resistances because it is time consuming and expensive, and the availability of the test is limited.

Polymerase chain reactions

Polymerase chain reaction (PCR) is a recent method of HP diagnosis and targets HP species-specific gene samples taken from blood, saliva, feces or biopsy tissues [32, 33]. The advantages of PCR are genotyping of HP and detecting antibiotic resistance genes and gene mutations [34-37]. The sensitivity and specificity of this method is 97% and 91.8%, respectively, even in peptic ulcer disease compared to RUT (47.7%) and histology (71.6%) [38].

Therefore, it can be diagnostic as well as investigative regarding the choice for appropriate therapeutic drugs. However, the inability to distinguish dead and living HP organisms is the main limitation of this method for confirming eradication. Furthermore, it may give false-positive results by detecting cDNA from non-HP organisms [39, 40].

Non-invasive methods

Urease breath test: (^{13}C and ^{14}C)

At present, the urease breath test (UBT) is considered the gold standard method of diagnosing HP infection in both pre- and post-eradicated cases [2]. There are two carbon isotopes used for UBT: ^{13}C -non-radioactive and ^{14}C -radioactive. Both methods are non-invasive, simple, highly accurate, and comparably expensive, and the ^{13}C UBT is usually not portable. However, the ^{14}C UBT "Heliprobe" is portable and simple, and diagnosis can be made in 20 minutes [41, 42]. Although ^{13}C UBT is a very reliable diagnostic test in children older than 6 years of age, it is unreliable in children below 6 years of age as well as in those who have taken antibiotics or acid suppressing drugs for 2-4 weeks prior to the test [43]. In partial gastrectomy patients, the lateral recombinant position provides a better test result of ^{13}C UBT [44]. The pretreatment ^{13}C UBT dose (15, 25, 50, 100 mg) has a sensitivity of 96-100% and specificity of 100%. Furthermore, in post-treatment cases, 15 mg of ^{13}C UBT has a sensitivity and specificity of 100% and 98.9%, respectively. Therefore, a low dose of 15 mg of ^{13}C UBT is as equally reliable as 100 mg [45, 46]. ^{14}C UBT is also the most reliable method of diagnosing HP infection in pre- and post-eradicated cases. The "Heliprobe", a ^{14}C UBT, has a sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of 92%, 100%, 100%, 84%, and 94%, respectively [42]. As ^{13}C UBT is non-radioactive, it has no radiation hazard, even in pregnancy. However, its non-portability is the main limitation. By contrast, ^{14}C is a radioactive isotope and has little risk of radiation hazard. Therefore, it is not usually recommended during pregnancy.

Fecal/stool antigen test (SAT)

The stool antigen test has very good diagnostic accuracy in pre- and post-treatment cases of H. pylori infections. A 4-8 week post-treatment evaluation has excellent results; however, an evaluation <4 weeks post-treatment after PPI is contradicted [4]. The pre-treatment monoclonal antigen technique performed better than the polyclonal technique in 8 studies (1399 patients), with a sensitivity of 96% vs 90%, specificity of 97% vs 94%, PPV of 96% vs 91% and NPV of 97% vs 85%, respectively. Additionally,

the monoclonal technique was comparatively better than the polyclonal technique in post-treatment 4-8 weeks after anti-secretory therapy. The sensitivity of SAT declined from 98% at baseline to 87% at 7 days and to 83% at 14 days after PPI treatment [4]. However, monoclonal stool tests are not as reliable as RUT, UBT, and histopathology for diagnosis of H. pylori infections [21].

IgG serology: Serum antigen marker

IgG antibodies serology is a very simple, widely available test for diagnosis of H. pylori infection. However, its persistence in post-eradicated cases calls into question its use in differentiating acute or past infections or performing post-treatment evaluation. The geographical variation in H. pylori strains can further decrease the reliabilities of the test, although using antigens pooled with different strains would increase its reliability [47, 48]. It may give a false positive test result in a low prevalence population due to its low specificity, which has to be confirmed by other reliable tests (SAT or UBT). However, it does not give false negative results in patients taking PPIs or antibiotics or in other clinical circumstances, such as atrophic gastritis, extensive intestinal metaplasia, and MALT. Additionally, particular virulence factors in H. pylori, such as CagA and VacA, can be identified by using specific antibodies serology. As the H. pylori antibody concentrations in saliva and urine are lower than that in serum, these sampling methods are questionable despite being easily obtainable [12, 49]. The serology test is not recommended in children because infected children may not achieve maximal H. pylori specific antibody levels until 7/8 years of age [43].

Management

The aim of treatment is to eradicate the H. pylori infection; reduce HP related complications, such as atrophic gastritis, peptic ulcer disease, and MALT lymphoma; and reduce the lifetime risk of gastric cancer in individuals with or without a family history, which is the major cause of mortality worldwide. The major indications for eradication in patients with HP positive tests are peptic ulcer disease with or without complications; low-grade gastric mucosa associated lymphoid tissue lymphoma, following resection of gastric cancer; dyspepsia; and patients with first-degree relatives of gastric cancer (Table 2) [4, 6]. Because antibiotic resistance is increasing day by day due to genetic variations or HP strains, the appropriate choice of multidrug regimens plays a prime role in the eradication rates. The factors that are included in choosing appropriate regimens are the prevalence of HP and gastric cancer, antibiotic

resistance, drug allergies and tolerances, previous history of treatment and outcomes, cost level, availability of bismuth, adverse effects and patient compliances [6]. The available treatment regimens and their eradication rates are based on intention-to-treat (ITT) and per-protocol (PP) analysis and are given in Table 3.

General measures

H. pylori infection is more prevalent in lower socioeconomic or developing nations and is transmitted by gut-oral or feco-oral routes. Maintaining good public health measures can play a major role in decreasing its prevalence. Washing hands thoroughly and consuming clean foods and water are additional general measures that should be followed. Eating particular foods, such as broccoli, green tea, and ginger, may have a beneficial effect. Additionally, these general measures only decrease its prevalence, and anti-*H. pylori* medications are necessary to eradicate infections.

Table 2. Indication for HP eradication

General recommendation	Strong recommendation
Functional dyspepsia	Peptic ulcer disease
Chronic gastritis with dyspepsia	Low grade gastric MALT lymphoma or Gastric MALT lymphoma
Chronic atrophic gastritis with erosion or intestinal metaplasia	
Long term use of proton pump inhibitor	
Family history of gastric cancer (first degree relatives with gastric cancer)	
After resection of early gastric cancer*	
Long term aspirin/ NSAID medication history with peptic ulcer disease	
Idiopathic thrombocytopenic purpura	
HP related diseases (gastritis, lymphocytic gastritis, gastric hyperplastic polyps, Menetrier disease, etc.)	
Iron deficiency anemia of unknown cause#	
Patients' wishes	
HP- <i>Helicobacter pylori</i> , MALT- Mucosa associated lymphoid tissue, NSAIDs- Nonsteroidal anti-inflammatory drugs	
*In Korea, post resection of early gastric cancer is a strong recommendation for HP eradication.	
#In China, iron deficiency anemia of unknown cause is also an indication for HP eradication	

Table 3. Treatment regimens and their eradication rates for *Helicobacter pylori* infections

Drugs	Treatment option	Eradication rates		Advantages	Limitation
		ITT (%)	PP (%)		
Standard therapy	Standard				
LAC7-14 [61]		85	91	Widely available	Failure in high resistance
LAL [63, 91]	2 nd -3 rd line	86-96		Alternative if failure of LAC	Not used in levofloxacin resistance area
MAE 7-14 days [50]	2 nd line	68-75.6	79.9-83.8	Alternative if Standard therapy failure	Higher resistances
Sequential therapy					
LA5+LCM5[61]		87	91.6		
EA5+ELM5 [47]	2 nd line	95.1	96.4	No effect of CYP2C19 polymorphism	Less effective in cases of resistance
		50(+MR)	97.7(-MR)		
		84(+GM)	95.1(GM)		
RA7+RMM7 [64]	1 st line	91.3	93.6	If bismuth unavailable	
RA7+RMC7 [64]		71.6	75.3		
OA5+OCT5	2 nd line	90	94.2	High resistance area	19% S/E
PA5+PMC5 [65]		89.2		High resistance area	
Concomitant therapy					
OACT5-14 [62]	2 nd line	85	95	Alternative to triple therapy	26.3% S/E
EBTL10[67]	2 nd line	78.9	87	Amoxicillin allergic cases	
EBTM10[67]	2 nd line	79.7	90.8	Second line in high resistance areas	
RRMB10 [73]	2 nd line	77.7	84	Used in treatment failure cases	
PAMC7 [65]	2 nd line	94.1		Alternative to ST, high resistance area	
EACM10 [66]	2 nd line	89.1	93.4	Alternative to ST.	
OA7+OCT7 [58]	2 nd line	82.7	95.7		
Rescue therapy					
ELA14 [70]	3 rd line	75	85.7	alternative to triple therapy failure	
OBAD7 [72]	3 rd line	91	92	High drug resistance area	
RABF7-14 [73]	3 rd line	89-90	85-90		
VAC7	Optional	89-92	91	P-CAB response better than PPI	Limited clinical trial
VAM7 [52, 53]		98			

ITT- intention-to-treat; PP- per protocol; ST- Sequential therapy; LAM14- lansoprazole, amoxicillin, and metronidazole for 14 days; LA5+LCM5- lansoprazole and amoxicillin for 5 days followed by lansoprazole, clarithromycin, and metronidazole for another 5 days; EA5+ELM5- esomeprazole and amoxicillin for 5 days followed by esomeprazole, levofloxacin, and metronidazole for another 5 days; (+/-)MR- with/without metronidazole resistance; (+/-) GM- with/without *gyrA* mutations; OACT- omeprazole, amoxicillin, clarithromycin, tinidazole; EBTL10- esomeprazole, bismuth subcitrate, tetracycline, and levofloxacin for 10 days; EBTM10- esomeprazole, bismuth subcitrate, tetracycline, and metronidazole for 10 days; RRMB10- rebepazole, rifabutin, minocycline, and bismuth subcitrate for 10 days; ELA- ecabet sodium, lansoprazole, amoxicillin bid for 2 weeks; OBAD7- omeprazole, bismuth subcitrate, amoxicillin, doxycycline for 7 days; RABF7-14- rebepazole, amoxicillin, bismuth subcitrate, furazolidone; VAC7- vonoprazan, amoxicillin, clarithromycin for 7 days; VAM7- vonoprazan, amoxicillin, metronidazole for 7 days

Antibiotic therapy (Specific therapy)

Standard therapy

Standard triple therapy (PPI+ amoxicillin+ clarithromycin) for 7 days is the worldwide followed first line treatment regimen. However, the PPI- based standard therapy fails in up to 30% of cases due to increased resistance to clarithromycin, subsequently reducing the eradication rate with standard therapy. Therefore, this therapy is usually recommended in low clarithromycin resistant regions. Azithromycin, a worldwide available macrolide, can be used instead; however, cross-resistance decreases the eradication rate. Increasing the duration of therapy to 10 or 14 days is suggested; however, 10 or 14 day use has controversial results and may increase antibiotic resistance [50]. To overcome this issue, sequential, concomitant or quadruple approaches have been alternatively used as a first line therapy [6, 51]. Recently, studies on a triple therapy containing a potassium-competitive acid blocker (P-CAB) (vonoprazan 20 mg+amoxicillin 750 mg+clarithromycin 200 mg/ metronidazole 200 mg bid*7d) compared to a PPI-based low dose clarithromycin triple therapy showed promising eradication rates of 89-98% [52, 53]. Therefore, P-CAB may be able to replace PPIs as an acid suppressing drug in the future.

Based on published clinical trials, quinolone-containing triple therapy is effective as a first line therapy and can be considered in populations with a clarithromycin resistance >15% and quinolone resistance <10%. Levofloxacin-containing triple therapy has eradication rates ranging from 86-96%, even in retreatment of clarithromycin-based triple therapy non-responders. Although the risk of quinolone resistance with urinary tract infections and respiratory infections is high [54-56], as a second line or third line option, PPI+ amoxicillin and levofloxacin for 10-14 days has significant benefits in eradicating *H. pylori* infection [57, 58].

Standard quadruple therapy is the regimen of choice in the case of standard triple therapy failure and availability of a bismuth subcitrate drug. Quadruple therapy has better eradication rates compared to triple therapy [12, 14]. The combination of ampicillin-sulbactam (ampicillin 225 mg and sulbactam 150 mg bid.) has equal or higher efficacy in quadruple therapy, so ampicillin-sulbactam can be considered in cases of amoxicillin resistant *H. pylori* strains [59].

Sequential therapy

Sequential therapy has significant benefits in eradicating *H. pylori* infections. There are various regimens that are used for 10-14 days. Commonly used sequential therapies and their eradication rates are presented in Table 3. The eradication rates with these therapies vary from 85-100%. The 10 day therapy has fewer side effects compared to the 14 day therapy, and there are no significant differences in eradication rates [57, 60]. Clarithromycin-based sequential therapy (lansoprazole, amoxicillin bid*5d, followed by lansoprazole, clarithromycin, metronidazole bid*5d) is not superior to 14 days of standard triple therapy in low clarithromycin resistance areas [61]. However, sequential therapy (omeprazole 20 mg, amoxicillin 1 gm bid*5d, followed by omeprazole 20 mg, clarithromycin 500 mg, tinidazole 500 mg bid* 5d) demonstrated eradication rates of 91.1% and 92.1% according to ITT and PP analyses, respectively, which was significantly better compared to the eradication rates with metronidazole [62]. In a multicenter trial on failed standard triple therapy, sequential therapy (esomeprazole 40 mg+ amoxicillin 1 mg bid*5d followed by esomeprazole 40 mg+ levofloxacin 240 mg+ metronidazole 500 mg bid*5d) showed a higher eradication rate of >95%. The eradication rates were significantly higher in the non-metronidazole resistance group (>97%) and non-*gyrA* mutation group (95.1% vs 84.6%). Additionally, the CYP2C19 polymorphism did not affect the eradication rates [63]. In comparing moxifloxacin-based (Rebeprazole 20 mg+ amoxicillin 1 gm bid*7d followed by Rebeprazole 20 mg+ metronidazole 500 mg+ moxifloxacin 400 mg bid*7d) versus clarithromycin-based sequential therapy, there were significant eradication rates of 91.3/93.6% (ITT/PP) with moxifloxacin-based therapy. Therefore, it can be very effective as a first line therapy with excellent patient compliance and safety compared to clarithromycin-based sequential therapy (eradication rates of 71.6/75.3%) [64].

Concomitant therapy

Concomitant therapy is another option for eradicating *H. pylori* infections in addition to standard therapy and sequential therapy. In these therapies, normally all four drugs are concomitantly given for 7-14 days. They have significant beneficial effects in eradication rates, although the side effects may occur for a longer duration [60]. The various regimens and their efficacies are presented in Table 3. In a multicenter prospective RCT conducted in a high clarithromycin resistant area, concomitant therapy (esomeprazole, 40 mg; amoxicillin, 1 mg;

clarithromycin, 500 mg; metronidazole, 500 mg bid*10d) had significantly higher eradication rates of 89% and 93.4% (versus 78.7%/82.8% of sequential therapy) according to ITT and PP analyses, respectively, despite drug resistances (metronidazole, 34%; clarithromycin, 27%; and dual drugs ,7.9%) on culture [9]. In another study, 7 days of concomitant therapy (pantoprazole, 40 mg; amoxicillin, 1 gm; clarithromycin, 500 mg; metronidazole, 500 mg bid) showed eradication rates of 94.1%, which was significantly higher compared to that of 7-day standard triple therapy (81.6%) and 10-day sequential therapy (89.2%) [65]. Additionally, 7-day concomitant therapy (Rebeprazole, amoxicillin, clarithromycin, metronidazole) showed eradication rates of 90.3% [57]. In another study with omeprazole, 20 mg; amoxicillin, 1 gm; clarithromycin, 500 mg; and tinidazole, 500 mg for 14 day, the eradication rates were 86.3% and 95% according to ITT and PP analyses, respectively [60], whereas the 5-day treatment showed eradication rates of 85.5% and 91.6% according to ITT and PP analyses, respectively [62]. Therefore, these therapies with 7-14 day durations can be an alternative to sequential therapy in high drug resistance areas and also in settings where bismuth is not available [9, 57, 62, 65, 66].

Ten-day bismuth-based second line quadruple therapies have comparably lower eradication rates. The study with EBTL (esomeprazole, 40 mg bid; bismuth subcitrate, 300 mg qid; tetracycline, 500 mg qid; levofloxacin, 500 mg OD* 10 d) showed eradication rates of 78.9% and 87% according to ITT and PP analyses, respectively [67]. In a high dose metronidazole study, EBTM (esomeprazole, 40 mg bid; bismuth subcitrate, 300 mg qid; tetracycline, 500 mg qid; metronidazole, 500 mg qid*10d) showed eradication rates of 79.7% and 90.8% according to ITT and PP analyses, respectively. Metronidazole-based quadruple therapy is cheap, safe and well tolerated. Therefore, this treatment could be an option for second line therapy for *H. pylori* infections in high resistance areas [67]. A bismuth-based PARB regimen (pantoprazole, amoxicillin, rifabutin, bismuth subcitrate) is the third line quadruple therapy in *H. pylori* eradication. A study with a PARB regimen (pantoprazole, 20 mg; amoxicillin, 1 gm; rifabutin, 150 mg; bismuth subcitrate, 240 mg bid) for 10 days demonstrated a significant therapeutic gain, with eradication rates of 96.6% compared to triple therapy without bismuth subcitrate (66.7%) [68].

A randomized trial of levofloxacin, 250 mg; omeprazole, 20 mg; nitazoxanide, 500 mg bid; and doxycycline, 100 mg qd (LOAD) for 7 or 10 days had eradication rates of 88.9% on LOAD7 and 90% on

LOAD10. This result demonstrated that the LOAD regimen is highly active in cases of treatment-naïve patients with HP infections. However, further large randomized controlled trials are needed to evaluate the efficacy of this regimen [69]. A meta-analysis showed treatment compared with doxycycline and tetracycline only, and there was no difference in eradication rates; however, compared with other regimens, there were higher eradication rates with doxycycline [70].

Hybrid therapy

A prospective randomized study with hybrid therapy of omeprazole, 20 mg, and amoxicillin, 1 gm twice daily for 7 days, followed by omeprazole, 20 mg; clarithromycin, 500 mg; and tinidazole, 500 mg twice daily for another 7 days, showed eradication rates of 82.7% and 95.7% according to ITT and PP analyses, respectively. The side effects were comparably less than those of 14-day concomitant therapy (22.7% vs. 26.3%) [60]. Another multicenter pilot study with a hybrid therapy of esomeprazole, 40 mg, and amoxicillin, 1 gm, twice daily for 10, 12 or 14 days plus clarithromycin, 500 mg, and metronidazole, 500 mg, twice daily for the final 7 days showed eradication rates of 95%, 95.1%, and 93.4% for the 10, 12, and 14 day therapies, respectively. This study concluded that in moderate to low clarithromycin or metronidazole resistance areas, therapy can be shortened to 10 or 12 days. However, further study is essential in moderate to high clarithromycin, metronidazole or dual resistance regions [71].

Rescue therapy

A study (n=74) was performed in patients with failed eradication of HP triple therapy (lansoprazole, 30 mg; amoxicillin, 750 mg; clarithromycin, 200 mg bid) in a Japanese population with rescue therapy (ecabet sodium, 2 gm; lansoprazole, 30 mg; amoxicillin, 750 mg bid) for 2 weeks. The study demonstrated eradication rates of 75% and 85.7% according to ITT and PP analyses, respectively [72]. The anti-HP effect of ecabet sodium has beneficial effects and can be an option for rescue therapy in standard clarithromycin-based triple therapy failure cases.

A rescue quadruple therapy that is tetracycline-based, RRMB (Rebeprazole, rifabutin, minocycline, bismuth subcitrate), in first line triple or sequential therapy and successive levofloxacin-based triple regimen failure cases showed an eradication of 77.7% and 84% according to ITT and PP analyses, respectively. This study concluded that a RRMB regimen can be an option in those who have failed at

least two previous regimens; however, a trial needs to be performed in a larger population [73]. A culture-guided rescue quadruple therapy using omeprazole, bismuth, doxycycline and amoxicillin for 1 week demonstrated eradication rates of 91% (ITT) and 92% (PP) despite high drug resistances (metronidazole, 100%; clarithromycin, 95%; levofloxacin, 31%; and tetracycline, 5%). Therefore, this amoxicillin and doxycycline-based quadruple regimen can be a good third line rescue treatment option [74]. Another rescue trial with furazolidone based quadruple therapy (rebeprazole, 20 mg; amoxicillin, 1 gm; bismuth subcitrate, 220 mg; furazolidone, 100 mg bid*14 days or furazolidone, 100 mg tid *7 days) had eradications rates of 89%/85% according to ITT/PP analysis at 14 days and 90% at 7 days [75].

Alternative therapies

Probiotics and phytomedicines in *Helicobacter pylori* infections

Probiotics and phytomedicines are alternative therapies and can reduce antibiotic resistance and increase eradication rates. The most commonly used probiotics are lactic acid producing micro-organisms, such as *Lactobacillus* spp. and *Bifidobacterium* spp. Phytomedicines include plant extracts or herbs, ginseng, green tea, red wine, flavonoids, broccoli sprouts, and garlic. The mechanisms of alternative therapies include (i) modulating the host immune response and normal gut microbiota, promoting host health; (ii) decreasing antibiotic associated side-effects; (iii) competing for nutrition and adhesion of cell receptors, leading to reduced colonization of HP; (iv) stimulating mucin production and stabilizing the gut mucosal barrier; and (v) inhibiting the urease enzyme [1, 76-78]. *Lactobacillus acidophilus* daily

supplementation for two weeks pre- and post-treatment with standard triple therapy (omeprazole+ clarithromycin+ amoxicillin bid) for 7 days demonstrated significantly higher eradication rates at pre-treatment (79.5%) and post-treatment (79.2%) than without probiotics (60.8%) [79]. Additionally, meta-analyses on supplementation with probiotics demonstrated comparably higher rates of *H. pylori* eradication in patients who supplemented with probiotics, and the total side effects (especially diarrhea) were also comparably less with probiotics [80, 81].

Antibiotic resistance

In the present context, increasing antibiotic resistance rates and decreasing eradication rates are the major problems in treating *H. pylori* infections. The higher rates of clarithromycin resistance in many countries have contradicted its empirical use in standard anti-*H. pylori* regimens. The overall worldwide resistance rates of metronidazole and clarithromycin are significantly higher followed by levofloxacin compared to other drugs (Table 4) [6, 82]. Therefore, the culture-guided therapy for *H. pylori* is currently essential, especially in high antibiotic resistance regions. Based on a European Medicine Agency evaluation of medicinal products that are indicated for the treatment of bacterial infections, 3 categories were defined according to susceptibility to the given antibiotic agents: 0-10% resistant- usually susceptible, 10-50% resistant- inconsistently susceptible, and >50% resistant- usually resistant. *H. pylori* falls into the second category [12]. In European countries, resistance to metronidazole (34.9%) was higher, followed by 17.5% with clarithromycin and 14.1% with levofloxacin. The clarithromycin and levofloxacin resistance rates were significantly higher (> 20%) in Western, Central and Southern European

countries compared to Northern European countries (<10%) [83]. In Asian countries, the metronidazole resistances are predominantly higher in Southeast Asian regions (100%) and coastal regions of China (>90%). Amoxicillin (33%) and clarithromycin (45%) resistances are high in India, levofloxacin resistance (30-38%) is high in China [6, 84, 85] and gatifloxacin resistance

Table 4. Antibiotic resistances according to geographical regions

Regions	Metronidazole	Clarithromycin	Amoxicillin	Quinolones	Tetracycline	Furazolidone
Asia[6] [57,58]	27-100%	3-45%	0-33%	3- 38%	4-7%	1-5% (China)
Least					(India)	
High	Taiwan 27% SEA 100%	Thailand 3% India 45%	Taiwan 0% India 33%	India 3% China 30-38%	China1-5%	
Europe [56]	34.9%	17.5%	0%	14.1%	-	-
North America	44.1%	29.3%	2.2%	-	2.7%	-
South America	53-82%	4-24%	2%	-	-	-
	Columbia 82%	Argentina 24%				
Africa [6, 82]	55-90% (Senegal 90%)	29.3%	65.6%	-	43.9%	-
Middle East [6]	73-100% Egypt 100%	0-9% Iran 9%	0-21% Iran 21%	2-5%	0-5%	9% (Iran)

SEA- Southeast Asia

(47.9%) is high in Japan [86]. In African nations, metronidazole resistances are predominantly high (92%), followed by amoxicillin (65%), tetracycline (43%), and clarithromycin (29%). In contrast to other geographical regions, amoxicillin and tetracycline resistances are significantly high in African nations [82]. In North American nations, the drug resistance rates were 44% with metronidazole, followed by clarithromycin (29%) and multidrug resistance (15%) [82]. In South American nations, metronidazole resistances (Columbia- 82%) predominated, followed by clarithromycin (Argentina- 24%) [6]. In Middle Eastern nations, metronidazole resistances (70-100%) were significantly higher in many countries, followed by amoxicillin (21%). In contrast, clarithromycin resistances were lower in these nations (0-9%) [6]. There were no reports found on the resistances of PPIs, ranitidine, and bismuth [82, 87].

Future advances

In recent years, researchers have improved the understanding of the innate and adaptive immune responses against *H. pylori*. However, the development of a successful vaccine producing immunity against *H. pylori* is still uncertain [88]. Anti IL-22 antibody injection efficiently killed *H. pylori* in an in-vitro mice model. IL-22 played a critical role in vaccine-induced protection by promoting the expression of anti-microbial peptides, including RegIIIb. In conclusion, urease-specific memory Th17/Th22 cells could constitute immune correlates of vaccine protection in humans [89]. In another mouse model, conjugating antigen vaccines with GEM (non-genetically modified *Lactococcus lactis*) particles could lead to promising oral therapeutic vaccine formulations against *H. pylori* [90].

Conclusion

As the eradication of *H. pylori* infection is essential to prevent its ultimate complication, gastric carcinoma, a proper diagnosis can be performed using simple, non-invasive methods, such as UBT and FAT. Eradication can be further improved by testing for drug sensitivities, especially in high drug resistance regions, with the help of invasively biopsied samples for culture and sensitivity. Therefore, appropriate treatment regimens can be conducted according to the geographical regions, even in high drug resistant areas. Recently, clarithromycin-based standard triple therapy has become not recommended in high drug resistant regions; instead, bismuth containing quadruple therapy is preferred. Currently, sequential or concomitant quadruple therapies have significant higher eradication rates even in single or multiple

drug resistant cases; therefore, these treatments can be recommended in standard triple therapy failure or if there is a contraindication to bismuth containing quadruple therapy. Additionally, probiotics and phytomedicines in addition to triple or quadruple/sequential/concomitant therapies would not only improve the eradication rates but also decrease the antibiotic resistance rates.

Abbreviations

HP: *Helicobacter pylori*; RUT: Rapid urease test; UBT: Urease breath test; SAT: Stool antigen test; RUT: Rapid urease test; IgG: Immunoglobulin G; BMI: Basal metabolic index; CagA: Cytotoxin-associated antigen A, T4SS: type IV secretion system; VacA: Vacuolating cytotoxin A; PAI: Cag pathogenicity island (PAI) strains; MALT: Mucosa associated lymphoid tissue lymphoma; PCR: Polymerase chain reaction; ITT: Intention-to-treat; PP: Per-protocol; vs: versus; CI: confidence interval; UGIB: Upper gastrointestinal bleeding; cDNA: circulating deoxyribonucleic acid; ¹³C: ¹³ carbon isotope; ¹⁴C: ¹⁴ carbon isotope; PPIs: Proton pump inhibitors; P-CAB: Potassium competitive acid blocker; CYP2C19: Cytochrome P450 2C19; RCT: Randomized control trial; EBTL: Esomeprazole, bismuth subcitrate, tetracycline, levofloxacin; EBTM: Esomeprazole, bismuth subcitrate, tetracycline, metronidazole; PARB: Pantoprazole, amoxicillin, rifabutin, bismuth subcitrate; LOAD 7 or 10: Levofloxacin, omeprazole, nitazoxanide, doxycycline for 7 or 10 days; RRMB: Rebeprazole, rifabutin, minocycline, bismuth subcitrate; GEM: non-genetically modified *Lactococcus lactis*.

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Competing Interests

The authors have declared that no competing interest exists.

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