Review Article

Current and Future Perspectives on *Helicobacter pylori* Antibiotic Resistant Mechanisms and Therapeutic Regimens

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Helicobacter pylori (*H. pylori*) is an important cause of gastritis, gastric and duodenal ulcer and even gastric cancer worldwide. Its resistance to antibiotics has greatly affected the eradication rates of the bacteria, making it more challenging to cure infected patients with first-line therapy. The first-line therapy consists of standard triple therapy, sequential therapy, concomitant therapy and bismuth-containing quadruple therapy, which usually involves proton pump inhibitor and many antibiotics such as clarithromycin, metronidazole, amoxicillin and tetracycline. Notably, *H. pylori* has been reported to show resistance to all of these antibiotics. The second-line therapies usually involve levofloxacin, and studies have also reported levofloxacin-resistant *H. pylori* strains. Novel therapeutic regimens including the use of probiotics in conjunction with antibiotics could be a promising new therapeutic regimen; however, further study regarding potential side effects, cost effectiveness and efficacy of the regimen is still needed. This review discusses current information on antibiotic resistant mechanisms and the novel therapeutic regimens for *H. pylori* infection.

Keywords: *Helicobacter pylori*, Antibiotic resistance, Therapeutic regimens

J Med Assoc Thai 2021;104(Suppl.2): S96-102
Website: http://www.jmatonline.com

Helicobacter pylori (*H. pylori*) has been identified as an important cause of gastritis, gastric and duodenal ulcer and gastric cancer[1,2]. Gastric cancer is the only cancer that is caused by bacteria, and antibiotic is the choice of treatment. Eradication of *H. pylori* to prevent gastric cancer was recommended by the Maastricht V/Florence Consensus, the Kyoto Global Consensus and the Toronto Consensus reports[3-5]. Current first-line therapy regimens of *H. pylori* are standard triple therapy, sequential therapy, quadruple bismuth therapy and concomitant therapy[6]. The second-line therapy was later developed to act as "rescue therapy" following the increase of *H. pylori* resistance strains. The treatment regimens include fluoroquinolone-based triple/quadruple therapy, tetracycline-levofloxacin quadruple therapy and high-dose dual therapy[7]. Therapeutic regimens of *H. pylori*, either first-line or second-line therapy, usually expose patients to more than one antibiotic and therefore lead to the development of antibiotic-resistant *H. pylori*, which greatly affects the eradication of the bacteria[8,9]. Consequently, many studies have attempted to find a way to improve the first-line therapy or find alternative regimens to overcome the problem. One of the novel regimens that shows promising results is the use of probiotics in conjunction with antibiotics to improve treatment tolerability and the eradication rate of *H. pylori*[10,11].

This article aims to summarize the current antibiotic resistance mechanisms of *H. pylori* against antibiotics used in therapeutic guidelines for better understanding of the underlying cause of treatment failure. Additionally, this review discusses the improvement of current therapeutic regimens and possible novel therapies that have been proposed to help in the eradication of *H. pylori*. The protocol of this research was reviewed and approved by the Human Research Ethics Committee, Chulabhorn Research Institute No. 063/2562.

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How to cite this article:
Hinthong W. Current and Future Perspectives on *Helicobacter pylori* Antibiotic Resistant Mechanisms and Therapeutic Regimens. J Med Assoc Thai 2021;104 (Suppl2): S96-102.
doi.org/10.35755/jmedassocthai.2021.S02.12562

H. pylori resistance mechanisms

The antibiotic resistance mechanisms of *H. pylori* discussed in this review focus on resistance to the specific antibiotics used in therapeutic guidelines for *H. pylori* treatment, including clarithromycin, metronidazole, amoxicillin, tetracycline and levofloxacin (Table 1).

Clarithromycin

Clarithromycin is a bacteriostatic antibiotic that binds to the 50S ribosomal subunit in bacteria, leading to inhibition of protein synthesis. Resistance of *H. pylori* to clarithromycin involves a point mutation in domain V of 23S rRNA in bacteria. This mutation inhibits clarithromycin binding to the ribosomal subunit. Mutations of adenine to cytosine at A2142G and A2143G positions are found to be

Table 1. Antibiotic resistance mechanisms *of Helicobacter pylori*

Antibiotics	Resistance mechanisms	References
Clarithromycin	Inhibition of antibiotic to binding to ribosome by point mutation in 23S rRNA	12 to 16
	Reduced accumulation of antibiotic by resistance-nudulation-cell division family of efflux pump system	17
Metronidazole	Mutation in rdxA gene (oxygen-insensitive NADPH nitro-reductase)	18 to 23
	Inactivation of frxA (NADPH flavin oxidoreductase) and frxB (ferrodoxin-like enzyme) genes	24, 25
	High expression of hefA gene (efflux pump system)	26
Amoxicillin	Loss of affinity between amoxicillin and PBP-transpeptidase by multiple point mutations in pbp1 gene	27, 28
Tetracycline	Reduce affinity of antibiotic and ribosome by substitution of an AGA with a TTC in the loop of helix 31 and deletion of G942 in Tet-4 site	33 to 37
	Removal of antibiotic from ribosome by soluble protein Tet(O)	38
	Decrease of membrane permeability and action of efflux pumps system	39
Levofloxacin	Prevention of antibiotic binding to enzyme by point mutations in gyrA and gyrB genes	40 to 44

frequently associated with the resistance. Point mutation at A2143G decreases *H. pylori* eradication rate more than A2142G or the substitution of adenine to cytosine at the 2142 position. The point mutations associated with low resistance to clarithromycin of *H. pylori* are T2182C, C2611A and T2717C[12-15]. Several other point mutations have been reported such as A2115G, A2144G, C2196T, T2117C, G2141A, G2224A, T2182C, T2183C, C2245T, T2289C, C2611 and T2717C, but none have yet been proven to be associated with clarithromycin resistance[14,16].

The efflux pump system is another mechanism related to macrolides resistance. The efflux pump family is found in several Gram-negative bacteria, and *H. pylori* has the resistance-nodulation-cell division efflux pump. Four of the RNA gene cluster (HP0605-HP0607, HP0971-HP0969, HP1327-HP1329 and HP1489-HP1387) in the efflux pump system have a role in the multidrug resistance of *H. pylori*[17].

Metronidazole

Metronidazole depends on the intracellular redox potential of bacteria to become active and damage DNA. This redox process is able to reduce metronidazole normally present in anaerobic bacteria but can also be found in *H. pylori*, which is a microaerophilic bacteria[18-20]. The main mechanism leading to metronidazole resistance in *H. pylori* is a mutation in the rdxA gene, which encodes an oxygen-insensitive NADPH nitro-reductase[20]. Several mutations are involved in inactivation of the rdxA gene including insertions and deletions of sequences, frame shift mutation and missense mutation[21-23]. Mutation of the rdxA gene was found to be associated with failure of eradication of *H. pylori*. Inactivation of the frxA and frxB genes encoding NADPH flavin oxidoreductase and ferrodoxin-like enzyme, respectively, were also found to be involved in *H. pylori* resistance to metronidazole both in the presence or absence of the rdxA gene[24,25].

A recent study reported that the efflux pump system seems to be directly involved in the resistance to metronidazole of *H. pylori*. The hefA gene encodes the outer membrane efflux protein. The expression of this gene was found to be significantly higher in resistant isolates of *H. pylori*, and the resistant isolate re-acquired susceptibility when hefA gene was knocked out[26].

Amoxicillin

Amoxicillin, a β-lactam antibiotic, interferes with the peptidoglycan synthesis of the bacteria by blocking the penicillin binding proteins (PBP), leading to a bacteriocidal effect on *H. pylori*. Several studies reported that multiple point mutations in the pbp1 gene, which lead to loss of affinity between amoxicillin and PBP-transpeptidase, are the main resistance mechanism in *H. pylori*[27,28]. Interestingly, β-lactamase production, which is considered to be the main penicillin resistance mechanism in other bacteria, was rare or found to be inactive in *H. pylori*[29-31]. Nevertheless, one study reported that a high level of amoxicillin resistance in *H. pylori* is associated with β-lactamase production, which suggests that this mechanism is not entirely absent from *H. pylori*[32].

Tetracycline

Tetracycline is a bacteriostatic antibiotic that acts by inhibiting codon-anticodon links at the 30S ribosomal subunit and prevents aminoacyl-tRNA to attach to the acceptor site (P site), thereby disrupting protein synthesis in the bacteria. The simultaneous triple point mutations by substitution of an AGA with a TTC in the loop of helix 31 at 965 to 967 position, which is the crucial part of the P site of the ribosome, is the major tetracycline resistance mechanism[33-35]. This reduces the affinity of the antibiotic

and ribosome by 24 to 52%[36]. Another mechanism is the deletion of G942 in resistant strains of *H. pylori*. However, this deletion occurs in the Tet-4 site, at which the affinity of tetracycline is significantly lower than at the primary P site. Therefore, the deletion of G942 could increase the bacterial resistance to tetracycline[37].

Other resistant mechanisms against tetracycline involve the soluble protein Tet(O), which helps remove the antibiotic from ribosomes and disrupts protein synthesis, decreasing membrane permeability and efflux pumps system; this lowers the accumulation of tetracycline in bacterial cells[38,39].

Levofloxacin

Levofloxacin is a fluoroquinolone antibiotic usually used in therapeutic regimens for *H. pylori*. Levofloxacin exhibits its bacteriocidal effect by binding to subunit A of DNA gyrase of the bacteria, thereby inhibiting DNA synthesis of the bacteria. A point mutation in the quinolones resistance-determining region (QRDR) of gyrA gene especially at position 91 (Asp to Gly, Asn or Tyr), position 87 (Asn to Lys or Tyr) and position 88 (Ala to Val) in *H. pylori* loci prevents the antibiotic from binding with the enzyme and makes the bacteria resistant to the antibiotics[40-42]. In levofloxacin-resistant isolates, 100% of the isolates were found to possess mutation in the gyrA gene at both 91 and 87 positions. Additionally, the mutation in gyrB gene has also been reported to potentially make *H. pylori* resistant to fluoroquinolones, and mutation at position 463 may be a new resistant mechanism of the bacteria[43,44].

Current and future H. pylori therapeutic regimens

Guidelines for eradication of *H. pylori* have been developed over the years, and clarithromycin triple therapy and bismuth quadruple therapy are usually recommended for the first-line of *H. pylori* eradiation. The Gastroenterology Association of Thailand published diagnostic and therapeutic guidelines for *H. pylori* with four recommendations: (1) triple therapy, sequential therapy, or concomitant therapy as the first-line therapy, (2) levofloxacin-amoxicillin triple therapy or bismuth-containing quadruple therapy as the second-line treatment, (3) antimicrobial susceptibility testing for suitable antibiotics and (4) use of probiotics as a supplementation to reduce the side effects from other drugs[45]. This review will focus on the recommendation from the Gastroenterology Association of Thailand and discusses each recommendation together with current research on improvements and developments of the regimens.

Triple therapy, sequential therapy or concomitant therapy

Triple therapy was recommended as standard proton pump inhibitor (PPI)-based triple therapy for 14 days and was shown to eradicate 85% of *H. pylori*. This regimen should not be used as the first-line therapy for only 7 days since the eradication efficiency reduces to 80%[46]. The American College of Gastroenterology (ACG) likewise recommends 14 days of treatment with triple therapy consisting of clarithromycin, amoxicillin or metronidazole and PPI in the region with low *H. pylori* clarithromycin resistance; however this approach should be avoided if the resistance is above 15%[47]. This regimen is successfully used in many parts of the world, with PPI, clarithromycin and amoxicillin as the most effective combination with 80 to 90% eradication rates[48,49]. Omeprazole is usually selected as the PPI in this regimen. Notably, several studies on gene polymorphisms of CYP2C19 (the enzyme involved in the metabolism of PPIs) showed that in individuals with polymorphisms, the use of esomeprazole, lansoprazole or rabeprazole as PPI is more effective than omeprazole[50-52]. A double dose of esomeprazole was also reported to significantly increase the cure rate of patients in high clarithromycin resistance areas[53]. Vonoprazan is a novel PPI that was found to a provide higher eradication rate over other PPI-based therapies such as lansoprazole triple therapy[54]. However, the cure rate of vonoprazan triple therapy was still unacceptably low for the clarithromycin-resistant strain of *H. pylori* (82%) when given for 7 days[55,56]. Further studies should be conducted regarding the efficacy of vonoprazan.

The 10-day sequential therapy consists of 5 days of lansoprazole and amoxicillin and 5 days of PPI, metronidazole and clarithromycin and was recommended in studies that reported an eradication rate of over 90%[57,58]. In the United States, this regimen is used as an alternative to the triple therapy[59]. Even though the sequential therapy has a higher eradication rate than the triple therapy of 7 to 10 days, the regimen was not superior to 14 days of clarithromycin triple therapy or 10 to 14 days of bismuth quadruple therapy and showed more side effects than standard therapy[6,60]. The sequential therapy can be modified to use levofloxacin instead of clarithromycin. The regimen consists of 5 to 7 days of a PPI and amoxicillin and 5 to 7 days of a PPI, levofloxacin and a nitroimidazole[47]. The modified sequential therapy was reported to have an eradication rate higher than that of standard sequential therapy (87.8% versus 71.1%)[61].

The concomitant therapy that consists of PPI and three antibiotics has a higher eradication rate than triple therapy[6]. Concomitant therapy consists of rabeprazole, amoxicillin, metronidazole and clarithromycin given for 10 days and was reported to have an eradication rate of 96.4% in Thailand[62]. The ACG also recommended 10 to 14 days of treatment with PPI, amoxicillin, clarithromycin and nitroimidazole to achieve a high eradication rate[47].

Levofloxacin-amoxicillin triple therapy and bismuth-containing quadruple therapy

The 10-day levofloxacin-amoxicillin triple therapy was recommended as a second-line therapy for *H. pylori* by The Gastroenterology Association of Thailand. However, this regimen is not recommended for patients with a history of chronic lung infection and who underwent regular fluoroquinolone treatment; this is because *H. pylori* may

rapidly develop resistance against antibiotics[45]. This regimen was superior than 7-day clarithromycin triple therapy if given for 10 to 14 days but has a similar eradication rate when given for 7 days[47,63,64].

The 10 to 24-day bismuth-containing quadruple therapy containing a PPI, bismuth subsalicylate and two antibiotics (for example, metronidazole and tetracycline) was recommended as an alternative second-line therapy in Thailand[45]. The ACG recommended using this regimen in regions known to have a high rate of clarithromycin resistance or in patients with a history of macrolide treatment. The regimen consists of a PPI or histamine-2 receptor antagonist, bismuth, metronidazole and tetracycline[47]. A study on the modified quadruple therapy (PPI, amoxicillin, metronidazole and bismuth subcitrate) versus bismuth-containing quadruple therapy was proposed and aims to provide the optimal treatment regimen for *H. pylori* in Korea; however, the work is still ongoing and requires time to prove the efficacy of the regimen[65].

Antimicrobial susceptibility test

Patients are likely to be exposed to more than one antibiotic in other therapies apart from bismuth-containing quadruple therapy such as concomitant therapy. These regimens potentially lead to further antibiotic resistance of *H. pylori*. Antimicrobial susceptibility testing to find the suitable antibiotics for *H. pylori* treatment and custom made therapy would be able to reduce the resistance rate and was recommended when two failures of other treatments were observed[45]. This recommendation is supported by a meta-analysis conducted by Chen et al that reported that the tailored therapies were superior to the empirical treatment with higher eradication rates in first-line therapy but showed no difference in rescue regimens[66]. Several current studies also reported no significant differences in empirical therapies and susceptibility-based therapy[67,68]. Additionally, side effects and cost effectiveness should be taken into account when making a decision on choosing the regimen.

Use of probiotics to reduce side effects from other drugs

The Gastroenterology Association of Thailand recommends the use of probiotics to compliment other treatments, while taking into account cost effectiveness analysis, side effects and prohibition of probiotic usage before making the decision[45]. Use of probiotics such as *Lactobacillus* and *Bifidobacterium* together with antibiotics to help eradicate *H. pylori* has become a topic of interest in many regions[69,70]. Anti-*H. pylori* activity of probiotics was reported for the *Lactobacillus johnsonii* strain, and *Lactobacillus salivarius* was reported to inhibit the attachment to epithelial cells in the human gastrointestinal tract[71,72]. This is considered as a direct effect of probiotics on *H. pylori* and could reduce the infection rate and protect against *H. pylori* colonization on gastric mucosa after treatment. Probiotics also help improve treatment tolerability of *H. pylori* therapies by reducing the adverse effects of antibiotic therapy such as decreased normal flora and overgrowth of antibiotic-resistant pathogenic bacteria[70,74-76]. The quintuple therapy, by adding lactoferrin and probiotics to a standard triple therapy, was proposed as a novel regimen for *H. pylori* treatment and improved the eradication efficiency and reduced side effects[77]. However, this regimen requires further study on subpopulations with resistant strains and the efficacy of different durations[10].

Conclusion

Many studies have been performed in an attempt to help improve the therapeutic regimens of *H. pylori* and even go further in post-treatment support with probiotics. Current therapeutic regimens, including triple therapy, sequential therapy, concomitant therapy and bismuth-containing quadruple therapy, still show acceptable eradication rates but the resistance to antibiotics from the regimens is also increasing in an alarming rate. The second-line therapy with fluoroquinolone-based was introduced and showed a higher eradication rate, but this should be used with caution because it could give rise to resistant strains and complicate the treatment. The novel regimens with probiotic supplementation are becoming the focus of many studies but the side effects and cost effectiveness should be considered when making a decision to use the regimen.

What is already known on this topic?

H. pylori has become resistant to many antibiotics involved in therapeutic guidelines as well as levofloxacin, which is considered to be a rescue therapy. The resistance mechanisms usually involve mutations in genes encoding the ribosomal subunit or enzymes crucial to the growth and survivability of the bacteria. Current therapeutic regimens for *H. pylori* eradication consist of PPIs, metronidazole, clarithromycin and tetracycline. The second-line therapy usually uses fluoroquinolone to improve the eradication rate in clarithromycin-resistant strain. Present studies have focused on improving the first-line therapy and also finding alternative regimens to overcome resistance and also improve tolerability of the treatment in patients.

What this study adds?

This article updates the information about the mechanisms of resistance in *H. pylori* against antibiotics used in current therapeutic regimens. Understanding more about the resistance mechanisms could lead to better comprehension of the underlying cause of treatment failure. Novel therapeutic regimens such as quintuple therapy, double dose therapy and modified triple therapy have been proposed as an alternative for first-line therapy but further study is needed to clarify the efficacy of each regimen. The use of probiotics in conjunction with currently used antibiotics showed promising results but more information is needed on the side effects, cost effectiveness and the efficacy of the regimens before determining whether the regimen is superior to the first-line therapy.

Acknowledgements

The author would like to express gratitude to the

Faculty of Medicine and Public Health, HRH Princess Chulabhorn College of Medical Science, Chulabhorn Royal Academy and Division of Research, Innovation and International Relations, Chulabhorn Royal Academy for providing support for this article.

Potential conflicts of interest

The author declares no conflict of interest.

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