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REVIEW

Importance of antimicrobial susceptibility testing for the management of eradication in *Helicobacter pylori* infection

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Abstract

The management of *Helicobacter pylori* (*H. pylori*) infection treatment differs from the common treatment protocol for other infectious diseases. Because culture- or molecular-guided approaches face several practical issues, such as the invasive procedures required to obtain gastric biopsy specimens and the lack of availability of routine laboratory testing in some places, *H. pylori* treatment includes the administration of two or three empirically selected antibiotics combined with a proton pump inhibitor rather than evidence-based eradication treatment. The efficacy of empirical therapy is decreasing, mostly due to increasing multiple resistance. Multiresistance to levofloxacin, clarithromycin, and metronidazole, which are commonly used in empirical treatments, appears to have increased in many countries. Mutations play a primary role in the antimicrobial resistance of *H. pylori*, but many different mechanisms can be involved in the development of antibiotic resistance. Determining and understanding these possible mechanisms might allow the development of new methods for the detection of *H. pylori* and the determination of antimicrobial resistance. A treatment based on the detection of antimicrobial resistance is usually more effective than empirical treatment. Nevertheless, such an approach before treatment is still not recommended in the Maastricht guidelines due to the difficulty associated with the routine application of available culture- or molecular-based susceptibility tests, which are usually administered in cases of treatment failure. The management of first and rescue treatments requires further research due to the steadily increase in antimicrobial resistance.

Key words: *Helicobacter pylori*; Antimicrobial resistance; Antimicrobial susceptibility testing; Susceptibility-guided therapy; Treatment management

Core tip: Eradication failure is of great importance in *Helicobacter pylori* (*H. pylori*) infection. Antibiotic resistance in *H. pylori* is widespread and increasing.

Therefore, understanding antimicrobial resistance mechanisms and detecting *H. pylori* antimicrobial susceptibility are important for guiding eradication regimens before the initiation of first-line therapy or alternative regimens for patients in whom repeated eradication therapies have failed. This manuscript presents an overview of the mechanisms of antimicrobial resistance and the methods that have been developed for the detection of resistance. It also highlights the contribution of antimicrobial susceptibility testing to the management of *H. pylori* eradication therapy.

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INTRODUCTION

Helicobacter pylori (*H. pylori*) is an important gastroduodenal pathogen of humans[1,2]. *H. pylori* infection is acquired in childhood and persists through life if a successful antimicrobial regimen is not performed[1,3-5]. Although most infected individuals are asymptomatic, *H. pylori* infection is responsible for the development of chronic gastritis, functional dyspepsia, and gastric or duodenal ulcers (1%-10%)[6]. More importantly, it is also linked to gastric adenocarcinoma (0.1%-3%) and gastric mucosa-associated lymphoid tissue (MALT) lymphoma (< 0.01%) in infected individuals[6-11]. *H. pylori* is defined as a Class I carcinogen by the World Health Organization and the International Agency for Research on Cancer[6,8,10]. In addition to gastrointestinal disorders, *H. pylori* also plays a role in extradigestive diseases, including immune thrombocytopenic purpura, unexplained iron deficiency anaemia, and vitamin B12 deficiency. The Maastricht Ⅳ and Ⅴ/Florence Consensus Reports suggest that "*H. pylori* should be sought and eradicated" for the management of these disorders[7-9,11-14].

The treatment regimens for *H. pylori* infection are generally based on the use of two antibiotics and one acid suppressant. Because *H. pylori* localizes on the acidic surface of the gastric mucosa, an acid suppressant (generally a proton pump inhibitor, PPI) is required for maintaining a constant pH and facilitating bacterial replication to increase the efficacy of antibiotics[3]. The standard triple therapy for *H. pylori* infection consists of a PPI in combination with clarithromycin and either amoxicillin or metronidazole[3,5,12-19]. Although the efficacy of this treatment regimen was high (> 90%) in the 1990s, the eradication rate of the triple therapy has fallen below the rate of 80% recommended by the Maastricht Ⅳ Consensus in recent years, mainly due to high antibiotic-resistance rates[6,9,11,13]. Many factors, such as antibiotic resistance, treatment compliance, dosage administered, duration of therapy, low gastric pH, cytochrome P450 2C19 (*CYP2C19*) gene polymorphisms, high bacterial load, impaired mucosal immunity and smoking, negatively affect the efficacy of the first-line triple therapy[6,8,20]. The eradication rates of the first-line standard therapy are 55%-57% in Western Europe, 74.5% in China, 84% in South Korea and 87% in Nigeria[8,21]. In cases of high clarithromycin resistance, bismuth-containing quadruple therapy (PPI, bismuth, tetracycline, and amoxicillin) or non-bismuth quadruple (concomitant) therapy has been recommended by more recent Maastricht Guidelines as the first-line therapy[12-14]. However, the success of these therapies remains controversial[22]. Other non-bismuth quadruple therapies, including the sequential and hybrid therapies suggested by the Maastricht Ⅳ/Florence Consensus Report, are no longer recommended by the latest Maastricht Ⅴ/Florence guideline as the first-line treatment in regions with high clarithromycin resistance. After first-line treatment failure, bismuth-containing quadruple and levofloxacin-based triple therapies are recommended as a second-line treatment[12-14]. In addition, bismuth-containing levofloxacin quadruple therapy is recommended as a second-line therapy by the Maastricht Ⅴ/Florence Consensus Report[12]. For cases of dual clarithromycin and metronidazole resistance (> 15%), only bismuth-containing quadruple therapy is recommended. If bismuth is not available, levofloxacin, rifabutin, and high-dose dual (amoxicillin and PPI) therapies are also suggested[12,14]. Culture- or molecular-based antimicrobial susceptibility testing should be considered for third-line treatment[12,13]. A combination of bismuth with antibiotics or rifabutin-containing rescue therapy in regions with high fluoroquinolone resistance is recommended by the recent guideline after clarithromycin-based first-line treatment and bismuth-containing quadruple-based second-line treatment failure[12]. In addition, the new three-in-one capsule drug, which contains bismuth, tetracycline, and metronidazole, could be important in this clinical setting and shows better efficacy (93% intention to treat) than the standard therapy[14]. A recent European multicentre survey performed in 2008-2009 reported primary resistance rates of 17.5%, 14.1%, and 34.9% for clarithromycin, levofloxacin, and metronidazole, respectively, but the tetracycline, amoxicillin, and rifampicin primary resistance rates were found to be $\leqslant$ 1%[6,16]. The quinolone resistance rates are high in several countries, particularly Portugal, Hungary, Austria, Belgium, France, Italy, and Germany[18]. Thus, high-efficacy therapy regimens are required for resistant *H. pylori* strains[6,16].

In routine clinical laboratories, the detection of antimicrobial resistance for *H. pylori* is mainly based on phenotypic methods performed after culture, including gradient diffusion susceptibility testing (E-test) and

the agar dilution method, which is preferred as a reference method. These methods have disadvantages, *e.g.*, results are not obtained until 48-96 h after inoculation of the agar plates[19,23,24]. The detection of *H. pylori* and the determination of antimicrobial resistance are very important before treatment if the clarithromycin resistance rates in the area have reached 15%-20%[25,26] or the metronidazole resistance rates have reached 40%[26]. When bacterial culture cannot be performed in a routine manner, molecular methods are alternative approaches for determining antimicrobial resistance[23]. The application of susceptibility testing prior to treatment does not always conclude with successful therapy, but the useless administration of inefficient antimicrobials, adverse drug effects, and the development of antimicrobial resistance can be minimized by pretreatment susceptibility testing[18]. The application of prior antimicrobial susceptibility testing has increased the eradication rates to 97% in Japan and from 84% to 95% in South Korea[21].

H. pylori treatment can be managed more accurately through antimicrobial resistance surveillance and the use of effective tests to determine antimicrobial susceptibility before treatment. The routine availability and applicability of *H. pylori* culture- and molecular-based antimicrobial susceptibility testing vary by country due to many factors, including a lack of experienced personnel, cost effectiveness, and test-related features, such as sensitivity and specificity. The antimicrobial resistance patterns of *H. pylori* vary from country to country and even from region to region. A stool sample-based molecular approach for detecting antimicrobial resistance in *H. pylori* might enable more convenient, time-saving methods that facilitate the applicability of susceptibility-guided treatment. The determination and understanding of the mechanisms that cause resistance can facilitate the development of new antimicrobial susceptibility methods that would allow identification of this resistance dynamism and guide optimized treatment prior to application of a standard eradication regimen[6-8,27].

This manuscript presents an overview of the mechanisms of antimicrobial resistance and discusses the importance of antimicrobial susceptibility testing for the management of the *H. pylori* infection treatment.

ANTIMICROBIAL RESISTANCE

Antimicrobial resistance is one of the most common reasons for treatment failure[28,29]. The prevalence of *H. pylori* antibiotic resistance varies by geographic area[30].

Clarithromycin is an acid-stable and the most common bacteriostatic, first-choice antibiotic used for the eradication of *H. pylori* infection[31]. The resistance rates to clarithromycin are high, varying from 16.4% in the United States, to 37.2% in Beijing, China, 7.7%-21.5% in Europe, 55.6% in Japan, and 12% in Latin America[8]. The clarithromycin resistance rate in Turkey was recently reported to equal 24.8%[32]. However, high clarithromycin resistance rates, including approximately 30% in Italy and Japan, approximately 40% in Turkey, and approximately 50% in China, were also reported in the current guideline[12].

Resistance to nitroimidazoles is the most commonly observed antimicrobial resistance in *H. pylori*. Metronidazole and tinidazole are the most frequently used nitroimidazoles for *H. pylori* treatment[25,33]. There is cross-resistance between metronidazole and tinidazole as well as ornidazole, which has not been used to treat *H. pylori* infection[25]. The resistance rates for metronidazole are 20.3% in the United States, 63.9% in Beijing, China, 28.6%-43.8% in Europe, 53% in Latin America[8], 37.1% in Asia, 92.4% in Africa, and 17.0% in Europe[30]. Thus, metronidazole resistance rates are highly variable[29]. In developed countries, metronidazole and related nitroimidazoles are frequently used for gynaecological infections, dental infections, and parasite-related diseases[29,34]. Although high metronidazole resistance rates are due to its common usage, the effect of high resistance on the eradication of *H. pylori* can be overcome by extending the duration time and increasing the administered dose[8,9,32].

High incidence of amoxicillin and tetracycline resistance in *H. pylori* infection was observed in certain geographic regions (*e.g.*, Italy, Brazil, El Salvador, India, and Lithuania) due to the non-prescription use of these drugs[33]. The amoxicillin resistance rates have been reported to equal 17.2% in Korea, 72.5% in India, 6.8% in China, 4% in Latin America, 2% in the United States, 65.6% in Africa, 11.6% in Asia, and 0.5% in Europe. The tetracycline resistance rate was generally < 1% but equaled 53.8% in India, 6% in Latin America, 3.5% in China, and 2.3% in Israel[21]. The rate of tetracycline resistance did not significantly differ among Europe (2.1%), Asia (2.4%), and United States (2.7%) but was higher in Africa (43.9%). Levofloxacin resistance was high in Beijing, China (50.3%)[8], although the levofloxacin resistance rates vary among countries: 14.9% to 38.6% in Japan and 11.9 to 6.8% in Taiwan. The recent resistance patterns revealed rates of 7.7%-18.6% in Europe, 31.3% in the United States, 41.3% in Vietnam, and 15% in Senegal and Latin America[8,21,30]. Primary rifabutin resistance was reported high in China (14.2%)[21] and Bulgaria (12%)[34] but low in Germany (1.4%) and England (6.6%)[30]. In Turkey, the overall resistance rates to clarithromycin, amoxicillin, metronidazole, tetracycline, and levofloxacin in *H. pylori* were reported in a systematic review to equal 24.9%, 1%, 33.7%, 3.5%, and 23.8%, respectively[32].

MECHANISMS OF ANTIMICROBIAL RESISTANCE

Antimicrobial resistance in *H. pylori* is due to point

mutations located on chromosome[33,35], whereas the mechanisms responsible for antibiotic resistance in other bacteria are associated with mutations in plasmids, transposons, or integrons[33]. Instead of horizontally transmitted plasmids, vertically transmitted point mutations are involved in antimicrobial resistance mechanisms. Transformation can occur when two different strains (mixed population) are simultaneously present in the stomach. As a consequence, a progressive increase in resistance is observed due to selection pressure[36].

Metronidazole resistance

Metronidazole is a synthetic nitroimidazole with bactericidal antibiotic function against microaerophilic microorganisms[11,25,33,37,38]. This drug, which is actively released into the gastric juice, is effective against *H. pylori* because its antimicrobial activity is only slightly affected by a low pH[33]. It is widely prescribed for the treatment of anaerobic bacterial and protozoal infections[10]. The antimicrobial activity of metronidazole is based on the reduction of metronidazole[29] by nitroreductases, such as pyruvate-flavodoxin reductase, *via* a single electron transfer within the cytosol of microorganisms, a mechanism that is particularly active in anaerobic bacteria following the production of toxic metabolites[10,11,37]. The intracellular redox potential of electron transport components is connected to the reactions of this reduction[39]. Metronidazole must penetrate into the bacterial cell for activation[10,25], and flavodoxin or ferrodoxin oxidized with electrons from the pyruvate oxidoreductase complex (POR) subsequently reduces the metronidazole nitro group in anionic radicals, such as nitroso- and hydroxylamine derivatives, to inhibit nucleic acid synthesis and attract the DNA of microbial cells, resulting in an impaired DNA helical structure and bacterial death[10,39]. The available molecular oxygen in the specific intracellular microaerophilic environment of *H. pylori* competes with metronidazole for electrons, leading to a re-oxidation of metronidazole radicals in a "futile cycle". The formation of superoxides results in DNA damage[10]. The distinct mechanism of metronidazole resistance in *H. pylori* has not yet been clearly explained, but some factors that might be relevant to metronidazole resistance mechanisms in *H. pylori* are transport deficiency, drug modification or export, loss or modification of the biological target, increased activities of DNA repair enzymes, increased expression of TolC homologous genes (namely *hp0605, hp0971, hp1327*, and *hp1489*) that upregulate efflux pump activity, reduced activity of nitroreductases, and an enhanced oxygen radical scavenging system[10,29,36,40].

An association has been reported between the overexpression of *H. pylori* superoxide dismutase (SOdB) due to introduction of a mutant-type ferric uptake regulator (Fur) and the development of metronidazole resistance. Mutation of the *furR3I* gene encoding the Fur protein affects the resistance to metronidazole by altering the cellular redox potential. The exact role of this mediation of metronidazole resistance by a scavenger system should be investigated further. Metronidazole resistance is previously reported to be related to one of the DNA repair enzymes in *H. pylori* encoded by the *recA* gene, and a significant decrease in metronidazole resistance was observed in a *recA*-deficient strain. In addition, overexpression of the *hefA* gene contributes to the acquisition of metronidazole resistance through the inhibition of metronidazole accumulation in *H. pylori*[10].

The above-mentioned mechanisms are important factors in metronidazole resistance. However, the predominant metronidazole resistance mechanisms result primarily from mutations in the oxygen-insensitive NADPH nitroreductase gene (*rdxA)* and/or the NADPH flavin oxidoreductase gene (*frxA*), which are putative metronidazole nitroreductase-encoding genes related to metronidazole resistance, and secondarily from mutations in the *fdxB* gene, which encodes a ferrodoxin-like protein[10,40]. High-level metronidazole resistance can be enhanced by inactivation of the reductase encoded by *fdxB*[41] (ferrodoxin-like protein-encoding gene) and *frxA* genes[39,41]. The *frxA* gene plays a role in converting metronidazole into an active compound that breaks down DNA[40]. Frameshift mutations, missense mutations, deletion of bases, and the presence of an insertion sequence (mini-IS*605*) have been demonstrated as four mechanisms of *rdxA* inactivation[37]. In addition, increased metronidazole resistance is acquired by disruption of *fdxB* together with inactivation of *rdxA*[10]. Nonsense and/or frameshift mutations causing premature truncation of both *rdxA* and *frxA* have been shown in all *H. pylori* strains with high-level metronidazole resistance. Intermediate metronidazole resistance in *H. pylori* strains is caused by a single premature truncation of either *rdxA* or *frxA*. The specific missense mutations in *frxA* causes low-level metronidazole resistance in *H. pylori* strains, and this effect is independent of any specific changes in *rdxA*. Nonsense and/or frameshift mutations in the *rdxA* gene have been reported in 68%-78% of metronidazole-resistant isolates[40]. However, other resistant strains showed no mutations in *rdxA*, and are identified by amino acid substitutions in the protein (R16H, A80T, A118S, Q197K, V204I, Y46H, P51L, A67V and C19Y) that appear to be key in the development of metronidazole resistance[10]. Because many factors contribute to the development of metronidazole resistance and some important mutations arising in the *rdxA* gene are independent of *H. pylori* resistance to nitroimidazoles, the development of molecular methods has not yet been confirmed[42].

Tetracycline resistance

Tetracycline is a bacteriostatic antibiotic that binds to the 30S subunit of the ribosome. It blocks

the binding of aminoacyl-tRNA to the ribosome, resulting in impaired protein synthesis and bacterial growth[33,36,37,43]. Tetracycline hydrochloride was the first effective therapy for *H. pylori* treatment, and the activity of tetracycline is independent of the acidity of the gastric mucosa[38]. Tetracycline resistance is achieved by either the overexpression of efflux proteins or changes in ribosomal proteins[33]. However, the main mechanism of tetracycline resistance in *H. pylori* is based on 16S rDNA mutations localized on the helix 31 region of the 16S rRNA molecule, the binding site of tetracycline[33,36,43,44]. A change in one nucleotidic triplet (AGA926 to 928→TTC) in the 16S rRNA gene has been associated with tetracycline resistance[33,36,43]. The AGA926 to 928→TTC triple-base-pair mutations cause high-level (⩾ 256 mg/L) resistance. Single- or double-base-pair mutations, such as AG926-927GT and A926G/A928C, are associated with low-level (1 mg/L and 4 mg/L) resistance[37,43,44]. Energy-dependent efflux plays a role in the resistance of clinical isolates of *H. pylori* to tetracycline[45].

Efflux pumps are a possible mechanism involved in tetracycline-resistant strains with no mutation in positions 926 to 928. Decreased tetracycline accumulation inside cells have been detected in these strains as well as in those with mutations[36].

Amoxicillin resistance

Amoxicillin is a bactericidal β-lactam antibiotic that belongs to the penicillin family[33,37,46]. Amoxicillin binds to penicillin-binding proteins (PBPs) to inhibit bacterial cell wall synthesis. Amoxicillin is released more easily into the gastric fluid than other penicillins[33] and exhibits pH-dependent antimicrobial activity. The minimal inhibitory concentration is adversely affected by increased pH. The highest amoxicillin concentrations are detected in the antrum, whereas lower levels are observed in the corpus and mucus layer[38]. The two most common mechanisms of β-lactam resistance in Gram-negative bacteria are due to the activity of β-lactamase and alterations in PBPs[10,33,37,47]. However, *H. pylori* appears to function differently. Although the *H. pylori* genome contains β-lactamase-like genes, considerable β-lactamase activity has not been detected in amoxicillin-resistant strains. Acquired or increased expression of β-lactamase enzymes appears to be unrelated to stable resistance to amoxicillin[10]. Although mutations of the *pbp-1A* gene or mutational changes in PBPs appear to be related to amoxicillin resistance in *H. pylori,* its resistance mechanism against amoxicillin is not completely understood[10,33,35,37,42].

No relationship was found to the β-lactamase activity that is traditionally involved in amoxicillin resistance in the two sequenced *H. pylori* strains[25,37]. The substitutions adjacent to the penicillin-binding motifs SAIK (368-371), SLN (433-435), and KTG (555-557) in PBP1 impart amoxicillin resistance in *H. pylori*[47]. Point mutations on the *pbp-1A* gene are associated with amoxicillin resistance in *H. pylori*[36,37,47]. Multiple amino acid substitutions in the transpeptidase region of PBP1, including Asn-562→Tyr, are necessary for resistance[47]. The amino acid substitution Ser-414 →Arg appears to be involved in and leads to blockage of penicillin transport[36]. Amoxicillin resistance in amoxicillin-tolerant strains is mediated by the absence of a fourth PBP (PBP4), PBP-D[29,33,36]. Amoxicillin resistance in *H. pylori* strains might be related to decreased membrane permeability. The MIC values can be further increased by point mutations in either *hopB* or *hopC* genes (encoding porin proteins) and are associated with mutations in the PBP1 genes[10,39]. Other mutations, including changes in *pbp2*, *pbp3, hefC,* and *hofH* as well as *hopB* and *hopC*, have been reported in amoxicillin-resistant *H. pylori* strains[10,11].

Fluoroquinolone resistance

Fluoroquinolones are bactericidal antibiotics that inhibit the activity of the topoisomerase Ⅱ (DNA gyrase) and topoisomerase Ⅳ enzymes necessary for DNA replication[10,25,33,37,42]. Mutations in genes that encode DNA gyrase *(gyrA* and *gyrB* genes) and/or topoisomerase Ⅳ (*parC* and *parE* genes) account for most fluoroquinolone resistance[10]. However, the *H. pylori* genome possesses genes for DNA gyrase but not topoisomerase Ⅳ (*parC* and *parE*)[42]. The fluoroquinolone resistance of *H. pylori* depends on point mutations in the quinolone resistance-determining region (QRDR) of the *gyrA* gene at positions encoding amino acids 86, 87, 88, 91, or 97[11,13,33,37,42,48,49]. Mutations in the *gyrA* gene of fluoroquinolone-resistant *H. pylori* strains are located at the codons for amino acids 86, 87 (Asn to Lys/N87K), 88 (Ala to Val/A88V), 91 (Asp to Gly, Asn, Ala, or Tyr/D91G, N, A, or Y), and 130 (Arg to Lys)[10,11,48]. The most frequent mutations are found at codons 87 and 91 of the *gyrA* gene[10,42]. Different amino acid substitutions related to fluoroquinolone resistance might be associated with the geographic differences in resistance. N87 *gyrA* has been most frequently detected in Japan, whereas D91 *gyrA* is most often observed in Hong Kong. Mutations at codon 91 might cause low-level resistance, but high-level fluoroquinolone resistance is caused by mutations at codon 87[10]. These point mutations are associated with a change in the MIC from ⩽ 0.25 to ⩾ 4 μg/mL[25,37]. Although a *gyrB* mutation has not been implicated in quinolone resistance[49], *gyrB* mutations have recently been detected at a rate of 4.4% in levofloxacin-resistant *H. pylori* strains, and 83.8% of resistant *H. pylori* strains have mutations on the *gyrA* gene. In addition to the recently discovered mutations at codon 463 of the *gyrB* gene, double mutations (D481E and R484K) in the *gyrB* gene have been reported in two levofloxacin-resistant *H. pylori* strains[10]. The overexpression of efflux pumps has also been found to be associated with fluoroquinolone

resistance in Gram-negative bacteria[45,48,49], but an important role for efflux pumps has not been reported in fluoroquinolone resistance in *H. pylori*[42]. However, some resistant strains do not have mutations in the QRDR of either *gyrA* or *gyrB*. Thus, investigating the role of mutations in other genes or the presence of plasmid-mediated resistance through cryptic plasmids was recommended. Cross-resistance toward levofloxacin, ciprofloxacin, and moxifloxacin in *H. pylori* and subsequent horizontal transmission of mutant resistance genes related to levofloxacin might be possible. Understanding the overall mutations and mechanisms of levofloxacin resistance is relevant for the quinolone-based treatment of *H. pylori*[10].

Rifampin resistance

Rifabutin is a spiro-piperidyl-rifamycin structurally related to rifampicin[3]. Rifabutin inhibits both RNA and protein synthesis by binding to the β subunit of the DNA-dependent RNA polymerase (encoded by the *rpoB* gene)[10,33,50]. Rifabutin is primarily used in tuberculosis treatment. However, the recent Maastricht guidelines state that rifabutin might be used as another candidate after two treatment failures for *H. pylori* strains with antimicrobial resistance to clarithromycin and levofloxacin[12,13]. Rifabutin resistance in *H. pylori* is due to point mutations in four different regions (codon 525-545, codon 585, codon 149 and codon 701) of the *rpoB* gene[10]. In particular, amino acid substitutions at codons 525-545 and 585 have been detected in all resistant laboratory *H. pylori* isolates and correspond to resistance in *Escherichia coli* and *Mycobacterium tuberculosis.* In addition to mutations at codons 525 and 544 or 585[10,11,33,37,50], a mutation at codon 149 (GTC→TTC) has been demonstrated to result in high levels of resistance. Other random mutations yield different levels of resistance through the addition of alternative amino acids. However, an exchange at codon 701 (CGC→CAH) results in low levels of resistance[10], and *rpoB* mutations are associated with high-level resistance to rifamycin [minimal inhibitory concentration (MIC) > 32 mg/L][50]. There was cross-resistance between rifabutin and rifampicin[11].

Clarithromycin resistance

Clarithromycin is frequently used as a macrolide antibiotic against *H. pylori* with bacteriostatic activity because of its unusual acid stability compared with that of other macrolides[10,51]. Its MIC value (as low as 0.016-0.15 mg/L) is lower than those of other antibiotics[51]. The antibiotic binds reversibly to hairpin 35 of domain Ⅱ and the peptidyl transferase loop of domain Ⅴ of the 23S rRNA molecule in the 50S ribosomal subunit[1,10,33,35,37,52]. This binding inhibits protein elongation through the premature release of peptidyl-tRNA from the acceptor site and thus effectively blocks bacterial protein synthesis. The antibacterial activity of clarithromycin is similar to that of other macrolides, but clarithromycin is better absorbed in the gastric mucus layer and is more acid-stable, making it more effective against *H. pylori*[10,33,35,37]. Resistance to clarithromycin in *H. pylori* is caused by several point mutations in the *rrl* gene encoding two 23S rRNA nucleotides, namely 2142 and 2143[25,37]. The mutations A2142G and A2143G are most often observed, whereas the A2142C mutation is less common[29,53]. Other point mutations have also been reported, but these mutations appear to be very rare, and their clinical importance has not been demonstrated[25,35,54-62].

Two transition mutations, A2142G and A2143G, and the transversion A2142C have been found in clinical specimens[17,37]. The frequency of A2142G and A2142C mutations is significantly higher in isolates with a higher MIC for clarithromycin (MICs > 64 mg/L), whereas the A2143G substitution is often found in isolates with a lower MIC (2-64 mg/L)[33,35,37]. Cross-resistance to macrolides could be due to a previous consumption of clarithromycin for the treatment of other diseases (*e.g.*, respiratory infections) or the intake of food products from antibiotic-treated animals[10]. As expected, clarithromycin resistance coincides with resistance to other macrolides. The A2142G and A2142C mutations are linked to high-level cross-resistance to all macrolides, whereas the A2143G mutation gives rise to high-level resistance to erythromycin and intermediate-level resistance to clindamycin and streptogramin[33,35,37].

H. pylori contains two 23S rRNA operons, and mutations are generally found in both copies. A mutation in one copy of the 23S rRNA might be easily transferred to the other 23S rRNA gene through efficient homologous DNA recombination under selective pressure, conferring higher levels of clarithromycin resistance[33,35,37,53,63].

Mutations in the *hp1048 (infB)* and *hp1314 (rpl22)* genes might be related to clarithromycin resistance in *H. pylori* due to their effect on MIC values. Downregulation of the iron-regulated membrane protein, Urease B, elongation factor Tu, and the putative OMP and upregulation of transmembrane proteins (HopT, HofC, and OMP31) have been associated with clarithromycin resistance in *H. pylori*[10].

Other mechanisms related to efflux pumps might be significant in the development of clarithromycin resistance[10,42].

ANTIMICROBIAL SUSCEPTIBILITY TESTING

The selection of appropriate and accurate antimicrobial susceptibility tests is important for the prescription of optimal antibiotics, the management of *H. pylori* treatment, the determination of patient-specific treatment, and epidemiological resistance surveillance[2]. Several methods, including phenotypic

and genotyping assays, are available for the detection of *H. pylori* antimicrobial susceptibility.

Phenotypic assays

Culture-based methods, including E-test, agar dilution, broth microdilution, and disc diffusion methods, are usually performed for antibiotic susceptibility testing of *H. pylori*[33,64]. These are applicable for all antimicrobial agents, which are tested through two-fold serial dilutions of various concentrations[29,37]. These methods offer the opportunity to determine the minimal inhibitory concentrations (MICs) of the antibiotics[29,33,64]. Agar dilution is a reliable technique and reference method for evaluating the accuracy of other methods according to the Clinical and Laboratory Standards Institute (CLSI)[23,33,37,38,40,48,50,64-66]. The following susceptibility and resistance MIC breakpoints for clarithromycin have been described: susceptible < 0.25 μg/mL; intermediate 0.25-1 μg/mL; resistant > 1 μg/mL[37,66]. There is no consensus regarding the levofloxacin, tetracycline, or amoxicillin breakpoints associated with the treatment of *H. pylori* infection[37,66]. The resistance cut-off value for metronidazole is 8 μg/mL, and this value was derived from the breakpoint values recommended by the CLSI for anaerobes[37]. Agar dilution could be performed reliably and is usually considered a reference assay compared with other techniques. Although its routine application is difficult[67], the method is adaptable for the testing of large numbers of strains[11]. The simplest, most cost-effective and most frequently used method for routine susceptibility testing is the disc-diffusion method. However, it has not been recommended for slow-growing microorganisms, such as *H. pylori*, due to unstable antibiotic patterns released from the discs[37,67]. E-test is a quantitative variant of the disc diffusion method[25,33,37] and is useful for slow-growing bacteria[67]. Its sensitivity and specificity are 45% and 98%, respectively[11]. Excellent correlation has been found between agar dilution and E-test MIC results for most antibiotics except metronidazole[23,29,37,67]. The rate of metronidazole resistance detected by an E-test might be overestimated by 10%-20% according to agar dilution-based results. This difference is associated with a lack of an anaerobic pre-incubation of plates in the E-test[37]. The MIC values obtained using the E-test can differ geographically. The most frequently used MIC breakpoint values for clarithromycin, metronidazole, amoxicillin, levofloxacin, and tetracycline are ⩾ 1 μg/mL, 8 μg/mL, 0.5 μg/mL, 1 μg/mL, and 2 μg/mL, respectively[15]. The MIC values of amoxicillin, clarithromycin, tetracycline, and metronidazole are 0.12 mg/L, 0.25 mg/L, 1 mg/L, and 8 mg/L, respectively, according to the European Committee on Antimicrobial Susceptibility Testing[2,15]. The correlation of these values with clinical outcome with respect to country should be assessed.

Although considered gold-standard methods, these techniques are time-consuming, and the results are not always consistent. Factors such as cell viability, inoculation size, incubation conditions, and growth media might affect the results[29,33,64]. *In vitro* susceptibility testing applies to only one antibiotic at a time, and possible *in vivo* synergies or other drug interactions in combination therapies cannot be identified through *in vitro* susceptibility testing[28]. Culture success might not be high (60%-70%)[68] due to various reasons, such as differences in transport conditions, materials (gastric biopsy or gastric juice), number of biopsies, and growth difficulties[7]. Culture and antimicrobial susceptibility testing of a biopsy sample from a single stomach site might not be representative. Different antimicrobial susceptibilities can be obtained from different parts of the stomach of the same patient, which negatively affects the success of susceptibility-guided therapy[69]. In addition, the use of gastric juice could be more reasonable for patients with an unsuccessful treatment because it represents almost the entire stomach area[68].

Genotypic methods

All phenotypic methods are slow, cumbersome, and fail in approximately 10% of cases due to biopsy contamination or growth failure of *H. pylori*. Nucleic acid-based methods are an alternative for the determination of antibiotic resistance. These methods are faster, independent of living bacteria, give reproducible results and are easily standardized[23,33,68]. These tests are available for the detection of clarithromycin, tetracycline, and levofloxacin resistance[33]. Molecular techniques for the detection of amoxicillin and metronidazole resistance have not been developed due to a lack of knowledge of the mechanisms involved in resistance to these antibiotics. There is no specific molecular method for the detection of rifabutin resistance, which can only be determined by sequencing[42]. Genotypic assays for antimicrobial resistance, such as PCR-restriction fragment length polymorphism (RFLP) and real-time PCR, dual-priming oligonucleotide (DPO)-based multiplex PCR, and DNA strip tests, might depend on the detection of A2143G in the 23S rRNA gene and Asn-87 or Asp-91 in GyrA[67].

Numerous PCR-based methods, including DPO-PCR, PCR-RFLP, real-time PCR, PCR-DNA enzyme immunoassay, mismatched PCR, hybridization, fluorescence *in situ* hybridization (FISH), and sequencing techniques, are now available for assessing the presence of *H. pylori* and clarithromycin resistance in *H. pylori* from biopsy specimens, gastric fluid, colonies, and even stool samples[70-74]. PCR methods based on the detection of point mutations have 98% sensitivity and 92% specificity[11]. RFLP is based on the presence or absence of a restriction site within the amplified DNA fragment. This assay allows detection of the previously mentioned 23S rRNA mutations using

the restriction endonucleases *Mbo*II (A2142G), *Bbs*I (A2142G), *Bsa*I (A2143G), and *Bce*AI (A2142C). As PCR-RFLP was initially unable to detect the A2142C mutation, a 3'-mismatch reverse primer PCR method (3 M-PCR) was developed[37,55-57,75]. Several real-time PCR hybridization assays have been developed. Real-time PCR techniques are a powerful advancement of the basic PCR method and were developed based on amplification of a fragment of the 23S rRNA gene of *H. pylori* following a melting curve analysis of biprobes and hyprobes[19,54,76]. Another molecular method is allele-specific PCR (ASP-PCR), which is based on the determination of single nucleotide polymorphisms in DNA samples through the identification of mutations without performing direct sequencing or digestion with restriction enzymes. The method allows the detection of clarithromycin and levofloxacin resistance. A previous study found a good correlation between ASP-PCR and both agar dilution and direct sequencing (*i.e.*, 100% concordance, sensitivity, and specificity for ASP-PCR of the 23S rRNA gene). The development of a new primer for the detection of N87I in GyrA in *H. pylori* has been reported[77]. In addition, line probe assays, including the commercially available GenoType HelicoDR assay, are DNA-based tests that can simultaneously determine multiple variants[11]. The standardized GenoType HelicoDR assay is rapid and allows the detection of both clarithromycin and fluoroquinolone resistance. Its sensitivity and specificity for clarithromycin are highly accurate (94%-100% and 86%-99%, respectively), whereas the corresponding values for levofloxacin are 83%-87% and 95%-98.5%[6].

The molecular detection of *H. pylori* and the determination of macrolide resistance from gastric biopsies can also be performed using FISH without DNA extraction and PCR[9,42]. This method is cultivation-independent, reliable, accurate, fast, and cost-effective[11,24,31,37,57]. It is also highly sensitive (97%) and specific (94%)[9,11], and outcomes are obtained from frozen[11] or formalin-fixed paraffin-embedded tissue sections within 3 h of endoscopy[42]. Coccoid forms of bacteria can also be detected using this method[42]. Nevertheless, degradation of the probe by proteases and nucleases found in the sample and poor diffusion of probes into the microbial cell wall might limit the success of this method. Peptide nucleic acid (PNA) probes involved in FISH (PNA-FISH) have been recently designed for the detection of bacteria, particularly in aquatic environments. This technique can be performed for the detection of clarithromycin resistance from colonies and histological gastric tissue preparations with a sensitivity of 80% and a specificity of 93.8%. PNA molecules are DNA mimics that are well-matched to DNA or RNA complementary sequences[11]. The PNA-FISH method uses fluorescently labelled PNA probes to identify DNA sequences on chromosomes. These small probes (13-18 nucleotides) penetrate the cell wall and are more resistant to proteases and nucleases than the DNA probes (> 18 nucleotides) used in FISH[11,42]. However, this method is not widely available for standardization[11]. A multiple genetic analysis system (MGAS) for the simultaneous detection of multiple antibiotic resistance is also available and suitable for *H. pylori* detection and screening[73]. *Helicobacter* species (*H. pylori vs H. heilmannii*) and antimicrobial resistance (macrolide and tetracycline) can be multiply detected using a microelectronic chip assay. The electrocatalytic detection of DNA sequences, which includes DNA hybridization and determines single base changes in target sequences related to clarithromycin resistance, such as the A2143C substitution, is another method[42].

Molecular-based susceptibility testing should be validated through comparison with the results of gold-standard traditional susceptibility testing in the same patients. Molecular-based methods might be superior to the gold standard, showing better identification of the mixed and multi-resistance defining susceptible populations[78]. These tests are easily standardized and give reproducible results. Moreover, they are faster than conventional culture-based assays, and the direct application of these techniques to gastric biopsy specimens allows the user to obtain data on the day of endoscopy[33,35]. However, high levels of mutations and recombination, frequent horizontal gene transfer, and the natural competence of *H. pylori* isolates result in genetic variability and can cause difficulty in the application of several classical genotypic techniques[79]. In addition, different geographic regions possess different patterns of mutations that lead to resistance[42]. Therefore, DNA sequencing provides a gold-standard reference method for mutation detection but is not technically feasible or cost effective for the routine laboratory determination of *H. pylori* resistance markers. Nevertheless, knowledge of nucleotide sequences has proven invaluable for validation of the various assays mentioned above, particularly when a resistant phenotype is not associated with any of the common mutations. A recent development in rapid sequencing based on the principle of pyrosequencing (a real-time DNA sequence analysis of short DNA stretches, 25-30 bp) has been applied to the rapid identification of *H. pylori*. The available data suggest that this new technology could offer an accurate and rapid technique for the sequence analysis of PCR amplicons because it provides easily interpreted results within hours[80].

CONTRIBUTION OF ANTIMICROBIAL SUSCEPTIBILITY TESTING TO THE MANAGEMENT OF *H. PYLORI* INFECTION

H. pylori infection is found in 50% of the world's population. Age and low socioeconomic status increase the prevalence of *H. pylori* infection[6]. Although disease development in response to *H. pylori* infection

is observed in only 10%-15% of infected individuals and is related to host genotype and strain-specific factors, the eradication of *H. pylori* infection represents an important strategy for recovering gastrointestinal (*e.g.*, gastritis, gastric and duodenal ulcers, gastric cancer, and MALT lymphoma) and extragastrointestinal (*e.g.*, idiopathic vitamin B deficiency, idiopathic iron deficiency anaemia, and idiopathic thrombocytopenic purpura) diseases. The eradication of *H. pylori* is therefore an important clinical need due to its positive effect on recovery from these diseases[6,7,12-14]. However, the treatment of *H. pylori* is challenging due to an increase in antibiotic resistance and a decrease in the efficacy of the standard empirical eradication therapy, including clarithromycin, amoxicillin, or metronidazole and PPI[7,8]. The success of the standard eradication therapy (first-line therapy) is thought to be lower than 85% if clarithromycin resistance is higher than 10%[81]. Several studies have confirmed that the eradication rate obtained with the standard therapy is 87%-92% in clarithromycin-susceptible *H. pylori* strains and decreases to 18%-21% in clarithromycin-resistant strains[82].

Antibiotic resistance in *H. pylori* is steadily increasing. The prevalence and rates of resistance in *H. pylori* vary among countries, regions and even time periods in the same area, as indicated by both Eastern and Western reports[6,7]. Therefore, the regular local surveillance of antibiotic resistance is essential for selecting the appropriate antibiotics for first-line *H. pylori* treatment in a given population when pretreatment antimicrobial susceptibility testing is not performed[6,8]. The use of a tailored treatment based on antimicrobial susceptibility testing is becoming more attractive due to its effect on the efficacy of treatment. This treatment has been extensively investigated because dynamic changes in resistance affect treatment outcomes[7].

Bismuth-containing quadruple therapy (PPI, bismuth, metronidazole, and tetracycline for 10-14 d) and non-bismuth quadruple (concomitant) therapy have been suggested by the Maastricht V/Florence Consensus Report as a first-line empirical treatment when the clarithromycin resistance rate is higher than 15%[12]. Otherwise, susceptibility-guided treatment is recommended by the Maastricht guidelines as the rescue therapy following first- and second-line therapy failures[12-14,27,69,81]. The Maastricht guidelines also recommend that, when the clarithromycin-based triple therapy is considered, culture and standard susceptibility testing can be performed either before treatment or after the first treatment failure in regions with high clarithromycin resistance if the tests are available and endoscopy is conducted but bismuth-based quadruple therapy has not been considered[7,12,13,83]. Given that the eradication therapy for *H. pylori* includes two or sometimes three antibiotics together with a PPI, multiple resistance to antibiotics makes administration of the correct therapy difficult and leads to an ineffective response to empirical therapy. Therefore, surveillance and antimicrobial susceptibility for all commonly used antibiotics facilitates effective therapy[7]. To optimize an eradication therapy for *H. pylori*, maximize the treatment efficacy, and prevent prolonged treatment, routine susceptibility testing can aid the prescription of appropriate antibiotic regimens prior to treatment for *H. pylori* in geographic areas with a high prevalence of resistant strains[84].

Several studies have reported that tailored treatment based on antimicrobial susceptibility testing can increase the efficacy of first-line and rescue therapies[6-9,27,85]. The eradication rates of susceptibility-guided treatments are generally higher than those of empirical treatment regimens[7,81,86]. Recent studies have also shown that susceptibility-based treatment improves the efficacy of the therapy used after first- or second-line treatment failure as well as the efficacy of first-line therapy (Tables 1 and 2)[26,73,82-84,87-92]. A meta-analysis of five randomized controlled trials concluded that the culture-based pretreatment success rates were 16% higher than those of various standard triple therapies used as the first-line treatment for *H. pylori* infection in 701 patients (per protocol 93% *vs* 76%)[6,9,82]. Another meta-analysis published by Chen *et al*[22] in 2016 included 13 controlled clinical trials comparing the eradication efficacies between tailored and empirical regimens and demonstrated that the efficacy of the first-line tailored therapy was higher than that of first-line empirical therapies. However, the eradication rates of tailored and empirical rescue therapies were not significantly different[22]. A recent systematic review and meta-analysis demonstrated that susceptibility-guided therapy prior to *H. pylori* treatment failure is a better eradication therapy than 7-10 d of empirical therapy; unfortunately, there is insufficient evidence or data regarding its role as a rescue therapy. The success of susceptibility-based treatment as a third-line therapy was not superior to the cure rates from reported empirical therapies, and the mean cure rate obtained with third-line therapies based on susceptibility testing was reported to equal 72%[27]. Draeger *et al*[93] demonstrated that the eradication rate obtained with susceptibility-guided therapy, including triple or quadruple therapy, against strains with dual or triple multiresistance to clarithromycin, metronidazole, and levofloxacin equalled 69.9% in 336 of 481 patients with at least one unsuccessful treatment. This cure rate is below the recommended cure rate (80%) and cure rates obtained in other studies. Susceptibility-guided therapy failure in 30% of patients might be obtained as a result of multiple eradication failures and the possible impact of various factors, such as grade of inflammation, gastric acid secretion, patient genetics, or polymorphisms in the *CYP2C19* gene or IL-1β. In the study, triple therapy including various combinations of amoxicillin, levofloxacin, rifabutin, and PPI was

Table 1 Summary of studies compared eradication rates of susceptible guided with emprical threapy for the first line eradication regimen

Ref.	Methods	Type of therapy		Patient number (*n*)		Eradication rates; ITT/PP (%)	
		SGT	Empirical therapy	SGT	Empirical therapy	SGT	Empirical therapy
Dong *et al*[73], 2015	E-test	Bismuth Quadruple Therapy (RpzBAC, RpzBAL, RpzBAF, RpzBAM, RpzBCM)	Quadruple Therapy (RpzBAC)	45	45	91.1/95.3	73.3/78.6
Park *et al*[87], 2014	Agar dilüsyon	P AC, PAM, PAL	PAC	57	57	94.7/96.4	71.9/73.2
Martos *et al*[82], 2014	E-test	OAC, OAM, OAL	OAC	55	50	94/94	67/72
Cosme *et al*[84], 2015	E-test	OAL, OAM, OAC	CT = OACM	122	181	94.2/95.1	87.2/88.7
Cosme *et al*[26], 2012	E-test	OAC, OBMT, OAL, OAM, OAR, OAD	OAC, OAM, OAL, OML, OMC	134	113	NR/88	NR/49
Zhou *et al*[92],2015	E-test	Rpz/EAC, Rpz/EATz	CT = EACTz, TTB = BEAC	350	350 TTB 350 CT	88.7/93.3	77.4/87(TTB) 78.3/87.4(CT)
Lee *et al*[88], 2013	PCR	RpzAC, RpzAM	RpzAC, RpzAM	218	616	80.7/91.2	69.5/75.9 (RpzAC) 71.1/79.1 (RpzAM)

ITT: Intention-to-treat; PP: Per protocol; O: Omeprazole; Rpz: Rabeprazole; P: Pantoprazole; L: Lansoprazole; E: Esomeprazole; C: Clarithromycin; A: Amoxicillin; L: Levofloxacin; F: Furazolidone; R: Rifabutin; D: Doxycycline; Tz: Tinidazole; T: Tetracycline; B: Bismuth; CT: Concomitant therapy; TTB: Triple therapy plus bismuth; M: Metronidazole; NR: Not reported.

Table 2 Eradication rates achieved with susceptible guided therapy as first treatment and rescue treatment

Line of therapy	Ref.	Type of therapy	Patients (*n*)	Methods	Tailored therapy eradication rates	
					ITT %	PP %
First Line	Liu *et al*[83], 2015	RpzBAC, RpzBAF	89	Real-time PCR	98 in RpzBAC group 92.6 in RpzBAF group	100 in RpzBAC group 94 in RpzBAF group
First Line	Sugimoto *et al*[90], 2014	RpzAC, RpzAM	153	PCR	96.7 (overall)	97.4 (overall)
Third line	Liou *et al*[89], 2013	Sequential triple	135	PCR and Agar dilution	80.7	82.6
Fourth	Fiorini *et al*[91], 2013	Triple (EAC, EAR)	236	E-test	NR	90 in EAC 88.6 in EAR

B: Bismuth potassium citrate; Rpz: Rabeprazole; A: Amoxicillin; C: Clarithromycin; F: Furazolidone; M: Metronidazole; R: Rifabutin; E: Esomeprazole; NR: Not reported.

administered to patients with dual clarithromycin and metronidazole resistance. Amoxicillin-rifabutin-PPI triple therapy, amoxicillin-PPI dual therapy, and quadruple therapy have been used for patients infected with clarithromycin-metronidazole-levofloxacin triple-resistant strains. Treatment with levofloxacin-rifabutin-PPI triple therapy has been found to have the highest cure rate (80.1%)[93]. In addition, a study performed by Mascellino *et al*[94] in 2015 reported that the eradication rates achieved with susceptibility-guided (by E-test) and empirical rescue treatments in pluritreated patients are 77% (48/62) and 84% (31/38), respectively.

However, a multicentre clinical trial conducted by the Taiwan Helicobacter Consortium found that the cure rate achieved with susceptibility-based sequential therapy applied after two eradication failures and detected using genotypic methods was 78.9% in patients administered clarithromycin-based sequential therapy[83]. In 2015, Kwon *et al*[95] also demonstrated the efficacy of susceptibility-based treatment as a second-line regimen strategy in 37 patients compared with an empirical second-line treatment in 171 patients. These researchers used an agar dilution method and 14 d of combination therapy using esomeprazole, bismuthate tripotassium dicitrate, metronidazole, and tetracycline (EMBT) or esomeprazole, moxifloxacin, and amoxicillin (MEA) based on susceptibility testing and reported that 14 d of susceptibility-guided therapy achieved higher eradication rates (87.8% by ITT, 100% by PP analysis) compared with 14 d of empirical EMBT (75.3%, ITT and 79.8%, PP) or MEA (70.8%, ITT and 72.4%, PP) treatment. Several studies have evaluated the effect of susceptibility-guided treatment on eradication rates and susceptibility testing prior to first-line therapy. The results suggest that the eradication rates are improved by antibiotic susceptibility testing[87]. Nevertheless, limited evidence is available for susceptibility-guided treatment[27], and its effects remain controversial[87]. In particular, the effect of performing antimicrobial susceptibility testing during rescue treatments is not

conclusive due to the limited number of studies and highly heterogeneous results, even though it has been recommended after second-line treatment failure in recent Guideline Consensuses[12,13,22,27,86]. Recent systematic reviews demonstrated that the overall results are insufficient to recommend the widespread use of susceptibility-guided therapies as a first-line or rescue treatment regimen for *H. pylori* treatment. Further studies demonstrating strong evidence-based outcomes are needed[27,86].

Tailored therapy arranged according to results from susceptibility testing and *CYP2C19* gene polymorphisms that affect the metabolism of PPI will always offer more satisfactory treatment outcomes than empirical treatment regimens in populations with resistant strains[7,78]. Culture- or molecular-based susceptibility testing and endoscopy have generally been required for susceptibility-guided therapy. These invasive tests, particularly culture and endoscopy based on biopsy specimens, often restrict the acceptability, effectiveness, and applicability of susceptibility-guided therapy[27]. Susceptibility-guided therapy cannot be applied at all locations due to a lack of endoscopy services and microbiology laboratories for performing antimicrobial susceptibility testing[7]. Although the culture of gastric biopsy specimens is initially applied as a gold-standard method for assessing antimicrobial resistance rates in *H. pylori*, the applicability and availability of culture-guided therapy are also limited by variations in culture success rates. These rates are influenced by many factors, including host-related factors, such as bleeding and the use of certain drugs, and methodology-related factors, such as the number of gastric biopsies, transport conditions, laboratory characteristics[7,83], and uncertainty in the interpretation of susceptibility[22]. In addition, low sensitivity, differences between *in vivo* and *in vitro* susceptibility, and time-consuming procedures reduce the feasibility of culture-guided approaches for treatment regimens against *H. pylori*[7,9,83]. Molecular-based methods allow the rapid detection and identification of mixed resistance. Non-invasive susceptibility characterization from stool samples, the detection of *CYP2C19* gene polymorphisms, and genotypic-guided treatment have been considered alternatives that are superior to phenotypic methods for the administration of tailored therapy[9,78,93]. Genotypic susceptibility-based treatment were demonstrated to achieve higher cure rates than culture-based treatment in a previous study[22]. However, a meta-analysis published in 2016[22] reported that pooled outcomes from 13 pretreatment tailored trials using either molecular-based (three trials) or traditional culture-based tests (10 trials) demonstrated higher eradication rates with susceptibility-guided therapies compared with empirical therapies. Culture-based susceptibility testing, including the determination of MICs using E-test, can detect resistance rates for all commonly used antibiotics (clarithromycin, levofloxacin, metronidazole, amoxicillin, tetracycline, and rifabutin). This method can be useful for selecting the proper combinations of antibiotics to that should be administered to patients who only have gastritis but are at risk for the development of atrophy, intestinal metaplasia, or gastric cancer because of family predisposition. In this nonurgent situation, the time-consuming process might be warranted. Molecular-based susceptibility testing is more useful for determining resistance rates and enhancing eradication rates in a short time or for a low bacterial load[6] in the case of diseases for which accurate and prompt treatment has an important effect, such as MALT lymphoma, the initial stages of gastric cancer, atrophic gastritis, and gastritis with intestinal metaplasia.

Cost-effectiveness trials are another important issue in the introduction of systematic susceptibility-guided *H. pylori* treatment. The prospective economic savings achieved with this approach compared with the standard therapy (achieving similar effectiveness) should be assessed. Cost-effectiveness trials depend on many factors associated with the efficacy of the *H. pylori* cure rate, including the eradication regimen, behaviour and patient compliance, number of prior eradication therapies, and geographical areas. The precise costs of endoscopic and biopsy procedures, materials used, administered drug regimens, and work hours of physicians and other professionals are linked to the cost-effectiveness of a culture-based approach. In this regard, antimicrobial susceptibility testing (particularly culture methods) might be more expensive or less commercially practical in some settings according to the target populations. The economic benefits of these approaches for first and subsequent treatments were assessed in recent trials[7]. Tailored triple therapy based on culture and susceptibility testing has been reported to be a more cost-effective, first-line treatment than the standard triple therapy. The economic savings of such approaches in 150 naive Italian patients were reported almost $5 per patient[6,7]. Similarly, Cosme *et al*[26] found that 10 d of omeprazole-amoxicillin-clarithromycin (OAC) eradication treatment, based on phenotypic susceptibility testing, was more cost-effective than empirical therapy, with savings of €95 per patient[7,26]. Cost efficacy can be increased by performing antimicrobial susceptibility testing in areas with high clarithromycin resistance (> 15%-20%)[6,26]. However, some controversial ideas complicate the cost efficacy of culture-based treatment for first-line therapy[6].

Although there is still no strict recommendation for antimicrobial susceptibility testing in routine practice, antimicrobial susceptibility tests for the management of *H. pylori* eradication, particularly prior to the initiation of eradication therapy, might provide many benefits[6,87]. Levofloxacin-based triple or quadruple therapy is recommended as a second-line treatment in the recent guideline[12] and is sometimes

used as a first-line treatment in regions with high clarithromycin resistance[6,8] but will not be effective in regions with high rates of multidrug resistance[87], including common clarithromycin, levofloxacin, and metronidazole resistance**.** Prior treatment failures result in the development of antimicrobial resistance[93] and an increase in resistance to antibiotics, particularly levofloxacin, resistance to which appears rapidly after usage[87]. A study conducted by Draeger *et al*[93] revealed that clarithromycin resistance increased to 60% after only one inefficient treatment and reached 80% after treatment failure. In addition, clarithromycin and metronidazole dual resistance and triple drug resistance (including quinolones, clarithromycin, and metronidazole) have increased to 65% and 15%, respectively[93]. In this regard, pretreatment susceptibility-guided therapy might reduce the rate of eradication failure by preventing the emergence and rapid acquisition of antimicrobial resistance and allowing a decrease in the incidence of metachronous gastric cancer in patients with gastric epithelial neoplasm[87]. An ideal low rate (approximately < 5%) of initial and overall eradication failure was recently achieved by Park *et al*[87] and Cosme *et al*[84] using pretreatment susceptibility-guided treatment. In addition, antimicrobial susceptibility testing before initial therapy might still allow the administration of clarithromycin-based standard therapy to patients with an *H. pylori* clarithromycin-susceptible strain in regions with high overall clarithromycin resistance[87]. Extending the duration of treatment or increasing the drug doses to overcome drug resistance (as in metronidazole resistance) might not be required for successful eradication through the application of pretreatment susceptibility-guided therapy, which would avoid the development of side effects and patient non-compliance[8,87]. Susceptibility-guided therapy as a third-line treatment might have limited efficacy due to the difficulty of culture and antimicrobial susceptibility testing after eradication therapy and the few available reliable drugs in the case of multiple resistance[87]. Methods with high sensitivity and specificity, such as molecular tests, might be more reasonable for the detection of resistance after eradication failure.

CONCLUSION

The Maastricht Ⅴ/Florence Consensus Report recommends a standard therapeutic regimen for the eradication of *H. pylori* in regions with low clarithromycin resistance and bismuth-based or concomitant treatment (non-bismuth-based quadruple) in regions with high resistance to clarithromycin[12,14]. Nevertheless, there are three major drawbacks that affect the treatment outcomes: possible lack of drug compliance, side effects, and development of antibiotic resistance. The application of susceptibility-tailored strategies might decrease the effects of these drawbacks on treatment outcomes. Tailored therapy for *H. pylori* based on antimicrobial susceptibility testing is still recommended by the Maastricht Guidelines as a third-line treatment after at least two empirical treatment failures due to the above-mentioned limitations of antimicrobial susceptibility testing (mostly culture-based testing) in routine clinical practice[7,12-14]. Susceptibility-guided *H. pylori* treatment has been demonstrated as an effective and useful alternative for reducing eradication failure. The need for a widespread implementation of susceptibility-based therapy as a first-line treatment in routine practice has not been fully demonstrated. The few available studies exploring the feasibility and effectiveness of such an approach have yielded heterogeneous results[27,86]. Thus, the role of susceptibility-guided therapy as a rescue strategy is restricted to the development of multiresistant strains and the difficulty of detecting low levels of bacteria after eradication failure. Practical, commercial, and logistical issues should be evaluated and addressed according to the target population and the clinical situation to determine the necessity of the therapeutic strategy prior to the application of susceptibility-guided *H. pylori* therapy[7,9]. The clinical diagnosis of patients should be taken into consideration when aiming to administer susceptibility-guided treatment for atrophic gastritis, intestinal metaplasia, the initial stage of gastric cancer, and MALT lymphoma in order to improve *H. pylori* eradication because the prescription of an effective patient-specific *H. pylori* treatment *via* antimicrobial susceptibility testing could prevent the development of these diseases and the exposure of the patient to repeated recommended standard strategies. Possible reinfection trials should also be considered. However, regular and regional surveillance of primary antimicrobial resistance is thought to be feasible and necessary for the management of *H. pylori* infection[6,13]. New pharmacogenomic tailoring therapy can be used in addition to susceptibility testing to determine *CYP2C19* gene polymorphisms and thereby select an effective PPI[7,9].

Culture-based susceptibility techniques have major limitations: they are time-consuming, costly, and invasive procedures, and they depend on many other factors. Molecular-based methods, particularly PCR-based and FISH techniques, can provide rapid and more accurate determinations of antimicrobial susceptibility through the detection of heteroresistant strains. However, molecular-based susceptibility tests are restricted to the detection of clarithromycin, levofloxacin, and tetracycline resistance, whereas culture-based susceptibility testing allows the detection of resistance to all commonly used antibiotics. The currently available molecular-based susceptibility methods were only designed to detect the common mutations causing resistance, and other possible mutational changes responsible for high resistance should be assessed and followed to ensure clinical compliance. However, a non-invasive, molecular

detection of resistance using stool samples might facilitate the application of susceptibility-guided treatments both before and after treatment due to the elimination of endoscopy dependency[6,7]. Antimicrobial susceptibility testing with phenotypic or genotypic methods is important for the ongoing assessment of antibiotics as well as for tailoring treatment by prescribing appropriate antibiotics, particularly for refractory *H. pylori* infection[6]. Ethical perspectives, such as approaches to patients, low prevalence of resistance (< 1%) in the population, and research design, should also be taken into consideration when planning further treatment regimens[78]. In conclusion, culturing of *H. pylori* and susceptibility testing should be performed after two treatment failures by obtaining gastric biopsy specimens whenever possible. The validated molecular tests, in addition to culture and susceptibility testing, should be performed for the management of *H. pylori* infection treatment.

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