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REVIEW

Antibiotic susceptibility, heteroresistance, and updated treatment strategies in *Helicobacter pylori* infection

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Maria Teresa Mascellino[1]
Barbara Porowska[2]
Massimiliano De Angelis[1]
Alessandra Oliva[1]

[1]Department of Public Health and Infectious Diseases, [2]Department of Cardio-Thoracic, Vascular, General Surgery and of Organ Transplants, Policlinico Umberto I, Rome, Italy

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Abstract: In this review, we discuss the problem of antibiotic resistance, heteroresistance, the utility of cultures and antibiotic susceptibility tests in *Helicobacter pylori* (*Hp*) eradication, as well as the updated treatment strategies for this infection. The prevalence of antibiotic resistance is increasing all over the world, especially for metronidazole and clarithromycin, because of their heavy use in some geographical areas. Heteroresistance (simultaneous presence of both susceptible and resistant strains in different sites of a single stomach) is another important issue, as an isolate could be mistakenly considered susceptible if a single biopsy is used for antimicrobial tests. We also examined literature data regarding eradication success rates of culture-guided and empiric therapies. The empiric therapy and the one based on susceptibility testing, in *Hp* eradication, may depend on several factors such as concomitant diseases, the number of previous antibiotic treatments, differences in bacterial virulence in individuals with positive or negative cultures, together with local antibiotic resistance patterns in real-world settings. Updated treatment strategies in *Hp* infection presented in the guidelines of the Toronto Consensus Group (2016) are reported. These suggest to prolong eradication therapy up to 14 days, replacing the old triple therapy with a quadruple therapy based on proton pump inhibitor (PPI), bismuth, metronidazole, and tetracycline for most of the patients, or as an alternative quadruple therapy without bismuth, based on the use of PPI, amoxicillin, metronidazole, and clarithromycin. The new drug vonoprazan, a first-in-class potassium-competitive acid blocker recently approved in Japan, is also considered to be a promising solution for *Hp* eradication, even for clarithromycin-resistant strains. Furthermore, there is growing interest in finding new therapeutic strategies, such as the development of vaccines or the use of natural resources, including probiotics, plants, or nutraceuticals.

Keywords: eradication, biopsies, rescue

Plain language summary

In this review, we discuss the issue of an important gastric pathogen, *Helicobacter pylori (Hp)*, which has been discovered quite recently in 1983. This microorganism is involved in several diseases such as gastritis, peptic ulcer, lymphoma, gastro-oesophageal reflux, and gastric cancer. This infection can be permanent in the absence of an appropriate treatment, and *Hp* intrinsic antibiotic resistance is an important obstacle to eradication.

Hp infection is widespread with about 50% of world population infected. In developing countries, especially in lower socioeconomic classes, the prevalence is higher (about 80%), whereas in the developed areas such as USA, Canada, Japan, and Western Europe, the prevalence is much lower (about 25%–30%). Therefore, it is crucial to identify effective therapies capable of curing *Hp* infection. We reviewed literature data searching for the best options to eradicate this pathogen that ensure both patients' compliance and the fewest side effects.

Correspondence: Maria Teresa Mascellino
Department of Public Health and Infectious Diseases, Policlinico Umberto I°Viale del Policlinico 155, 00161 Rome, Italy
Tel +39 6 4997 0880
Fax +39 6 4997 2628
Email mariateresa.mascellino@uniroma1.it

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Introduction

Helicobacter pylori (*Hp*) is a Gram-negative mobile bacillus, difficult to be cultured, which has been reported to be genetically extremely variable, and this heterogeneity is proposed to be involved in its ability to cause different diseases, detrimental and nondetrimental chronic infections.[1,2] As a matter of fact, *Hp* is involved in a wide variety of infections such as chronic active gastritis, peptic ulcer disease, gastric carcinoma, and mucosa-associated lymphoid tissue (MALT) lymphoma. Furthermore, epidemiological and eradication studies have demonstrated a causal relationship between *Hp* infections and endothelial dysfunction leading to vascular diseases.[3,4]

Usually, the colonization occurs primarily during childhood, especially in the developing areas, predominantly in the same family as a consequence of cohort effect.[5] This colonization is widely asymptomatic even if a long-lasting infection can be established in some subjects. Although the prevalence of *Hp* seems to decrease in some parts of the world, the presence of this infection remains high in some populations ranging from 25% to 86%, depending on the areas under consideration. According to the Center for Diseases Control in USA (www.cdc.gov/ncidod/dbmd/hpylori.htm), about 50% of the world population is infected with *Hp*.[6–8] Generally, the prevalence of infection is more closely correlated with socioeconomic conditions than with geographic origin. For what concerns industrialized countries, the prevalence is 20% of the population under 40 and 50% of people over 60, whereas it is rare in children. A low socioeconomic status and a large flux of immigrants represent risk factors for the infection in these settings. On the contrary, in developing countries, infection involves the majority of the adult population (about 80%) and 10% of children from 2 to 8 years old. The prevalence of *Hp* infection in the white population of USA and South Africa is almost comparable to that of Europe, whereas other ethnic groups have double prevalence values.[9] More virulent strains (CagA + and VacA +) have been found in the general male population in the UK reaching up to 44% of the tested individuals and even more in a previous epidemiological survey in Italy regarding a population of military students.[10,11]

Infection is virtually lifelong in the absence of treatment, implying that evasion of the host response is efficient.

Hp antibiotic resistance is a primary hurdle to achieving eradication. Treatment regimens that have been used over the past decade are declining in efficacy, and the treatment of this infection is bedeviled by drug-resistant strains. The leading causes of treatment failure are antimicrobial resistance and nonadherence to therapy (poor patient compliance).

Hp is described as a microorganism that can easily acquire resistance to antimicrobial agents leading to increased prevalence of antibiotic-resistant strains, thus reducing success with traditional therapies.[12] The acquired resistance can be due either to antibiotic consumption in individual lifetime or to repeated therapeutic attempts.[13] Susceptibility testing should be then the method of choice in guiding the most appropriate cure.[14]

The goal of *Hp* therapy should be the achievement of eradication in about 90% of treated patients but this result is hardly reached.[15]

The aim of the present review is to discuss the results of the sensitivity in vitro of the most common antibiotics, the presence of heteroresistance (HR) in different infected parts of the single stomach, the value of cultures and antibiotic susceptibility tests in the eradication rate, as well as updated treatment strategies.

Susceptibility to antibiotics

The most common antibiotics used in *Hp* treatment are the following: metronidazole (MZ), clarithromycin (CLA), amoxicillin (AMX), tetracycline (TE), levofloxacin (LEV). The resistance rates vary widely depending on geographical areas, so that therapy should be tailored according to regional antibiotic resistance patterns and individual characteristics. At the beginning of the 1990s, a low prevalence of CLA resistance was detected ranging from 1% to 8%, whereas MZ resistance resulted to be stable over time ranging from 20% to 40%. The resistance to AMX resulted to be very low (1%–3%).[16–18] In the last years the prevalence of CLA and MZ secondary resistance has become very high (67%–82% for CLA and 52%−80% for MZ).[19]

The patterns of resistance to antimicrobials may change over time, considering that in countries where CLA resistance is progressively higher, there is a subsequent use of MZ-based therapies, which leads to an increase in MZ resistance. *Hp* may acquire resistance to rescue antibiotics during the course of eradication therapies or treatments of other bacterial infections, thereby becoming multiresistant and difficult to remove.[15,20]

Bacterial antibiotic resistance can be classified as intrinsic or acquired resistance; the first is a genetic property for most bacterial strains and typically evolves regardless of clinical use of antibiotics; the latter implies that a susceptible organism has developed resistance to antimicrobial agents to which it was previously susceptible.[21,22]

A) Resistance to MZ: MZ resistance is due to point mutations regarding *rdxA* and *frxA* genes that prevent intracellular

activation of the drug by inhibiting the nitroreductases, which activates the prodrug (a synthetic nitroimidazole) in its active form within the cytosol of the bacterial cells.[23] MZ resistance is a frequent issue in various geographical areas because of epidemic diseases (eg, amoebic, gynecological, and anaerobic infections) endemic in different countries. In vitro results often do not correlate with in vivo efficacy. The E-test has been established as a reliable method for measuring antibiotic resistance to all antimicrobials in vitro, except for MZ for which it overestimates the resistance. This discrepancy between in vitro MZ resistance and treatment outcome may partially be explained by changes in oxygen pressure in the gastric environment, as MZ-resistant *Hp* isolates become MZ-susceptible under low oxygen conditions in vitro.[24] The role of these inactivating mutations on the reversibility of MZ resistance under low oxygen conditions is well established. In fact, it has been found that under anaerobic as well as microaerophilic conditions, all isolates lose their MZ resistance, while no effect is seen with other antibiotics used in *Hp* treatment such as AMX, TE, and LEV.[24] The resistance to MZ is quite high in almost all studied countries. Li et al[25] found 75.2% of resistance rate in a population of children in People's Republic of China. In Morocco, the rate was 40%, in the USA it ranged from 20% to 40%, in central Italy (Abruzzo) it was 34.69% in gastric antrum and 42.16% in fundus.[26–28] Only a multicenter study conducted in Northeast Italy by Pilotto et al[29] found a resistance rate as low as 14.9%. In any case, a higher resistance rate has been detected in developing countries (50%−80%) such as Mexico (about 77%).[30] In Japan and in Canada the prevalence was 9%−12% and 18%−22%, respectively.[31,32] These differences are probably due to an extensive use of MZ in each country. In fact, an earlier use of this drug is associated with an increase in MZ resistance.[33]

B) Resistance to CLA: CLA resistance is due to point mutations in the 23S rRNA genes (ie, A2143G and A2142G). These mutations prevent the macrolide from binding to the 50S ribosomal subunits.[34] Resistance to CLA has been studied on a large scale because of the fact that it has been considered the antibiotic of choice over the last years. The local pattern of *Hp* resistance to CLA results to be crucial in each country, taking into account that in countries where CLA resistance is above 15%–20%, this drug should not be used. CLA-resistant *Hp* has been extensively studied: its prevalence has become increasingly higher in many geographical areas. Resistance to CLA depends on the local *Hp* seropositivity rate. This means that in regions with a low prevalence of *Hp*, antibiotic resistance seems not to increase over time, whereas in regions with a high prevalence of infection, a high increase of antibiotic resistance related to the rise of *Hp* seropositivity is detected.[35,36] For instance, in People's Republic of China, an increase of CLA resistance from 15% in 2000 to 53% in 2014 was accompanied by a rise in seropositivity rates from 65% to 83%.[37–39] In contrast, in a multicenter retrospective study of children population in People's Republic of China, the pattern of *Hp* antibiotic resistance showed no significant changes in the resistance rates to CLA, AMX, furazolidone, and MZ over 7 years. The age of children slightly affected the resistance rates to CLA and MZ.[25]

Similarly in Japan, an increase in CLA resistance ranging from 1.8% in 1996 to 27.1% in 2008 has been described.[36] In Korea, CLA resistance increased from 11% in 2005 to 60% in 2009.[40] In Morocco (Rabat) the prevalence of primary resistance to CLA was 29% with 2% of strains showing double resistance to MZ and CLA at the same time.[26] In Central Italy, Di Giulio et al[28] performed a study involving nine antibiotics, which showed an overall CLA resistance rate of 72.44% in gastric antrum and 72.8% in fundus, concluding that the high rate of resistance to CLA and also to MZ (34.69% in antrum and 42.16% in fundus) as well as to quinolones (42.85% and 53.01% in antrum and fundus, respectively) might reflect their overuse. In the USA there is an increase of CLA resistance as well.[27] In a study on a pediatric population in the USA, the resistance rate was as high as 50%.[41]

The varying distribution of CLA resistance in different geographical areas raises the issue of an accurate study of the local situation of antibiotic resistance in order to better guide treatment regimens.

C) Resistance to AMX: Resistance to AMX is due to alterations of penicillin-binding proteins and to decreased antibiotic membrane permeability in the cytosol of bacterial cells (active efflux pump that excretes drugs).[42] AMX resistance results to be very low in most countries being <2% in Europe (Germany and the Netherlands) and higher (up to 38%) in Asia and South America.[43–46] In People's Republic of China it has been detected in only 0.06% of the population.[25]

D) Resistance to TE: The mechanism of TE resistance is based on the inhibition of protein synthesis through binding to the 30S subunit of ribosomes, thus blocking the synthesis of nascent peptide chains.[47] Resistance

to TE is very low (0.7%–1%) or even absent in most countries.[48,49]

E) Resistance to LEV: Point mutations of gyrase A coding for DNA gyrase are involved in LEV resistance.[50] There is a limited number of studies evaluating susceptibility to LEV. In Italy LEV resistance has been reported to be 22%–24% similarly in Portugal.[51,52] In People's Republic of China, in a multicenter, retrospective study of Chinese children, the resistance rates were lower, accounting for 6.7% of the study population.[25] LEV resistance rate of 11% was detected in Morocco by Bouihat et al[26] in a multicenter study of patients referred for gastroduodenal endoscopy who had never been treated previously. Anyway, there is concern that LEV resistance may increase in many countries.[53–55]

Taken together these data show that resistance to different antimicrobial agents varies greatly all over the world based on comorbidities, previous diseases, age, socioeconomic conditions, the number of eradication therapy cycles, the use and overuse of antibiotics, as well as the period of time in which the study was conducted. It is important to highlight all these variables in order to obtain a general view of the current situation in each country.

In our research conducted at the University Hospital in Rome, 61 strains were isolated from 50 patients affected with pangastritis who underwent at least two or more eradication cycles (up to nine). We have observed a higher resistance rate compared to other national studies performed in patients with different pathologies and no previous therapeutic regimens. As shown in Table 1 (Mascellino, unpublished data, 2013), the values of minimum inhibitory concentrations (MICs) of the tested antibiotics determined by E-test and relative rates of resistance regarding our patients are reported. Resistance to MZ was up to 82% and that to CLA reached 54%. These strains appear to be highly resistant to MZ with three strains showing an MIC value >256 mcg/mL. AMX is the most effective antibiotic as well as TE (5% and 7% of resistance, respectively). Resistance rate to LEV was 25%. Double resistance to MZ and CLA, which can be demonstrated in about half of the strains under study, may lead to a worse course of the disease and poor eradication outcome.

We have detected higher resistance rates compared to other studies, especially for CLA and MZ. In fact, in one survey taking into consideration a Polish population studied in 2014 (adult symptomatic patients with primary infection), among 50 *Hp* strains, 12 (24%) were resistant to CLA, 21 (42%) to MZ, and 4 (8%) to LEV. Examined strains were fully susceptible to AMX and TE. Moreover, the authors found a different sensitivity to CLA and MZ, according to patients' age (the highest CLA resistance was found in younger individuals, whereas the resistance to MZ increased with age).[56]

The discrepancy between the results of our preliminary research and the Polish study may be due to the differences between the study populations. In fact, in our case, patients with previous eradication failures were taken into consideration. In this setting, strain isolates can acquire higher resistance, considering that pangastritis patients are the most difficult to cure. In fact, in this selected population, *Hp* infection is quite characteristic, as the bacteria are capable of colonizing the entire stomach in the condition of reduced acid secretion. Thus, the mechanisms of virulence and persistence may be different compared with patients with normal acid secretion.[57]

Heteroresistance

HR is defined as the coexistence of susceptible and resistant isolates in the same patient for the same antimicrobial agent. This is a common occurrence in *Hp* population and can be explained either as the result of multiple infections (unrelated isolates) or as the presence of susceptible and resistant variants of the same strain (related isolates). In the latter case, HR has been described either as intradistrict when susceptible and resistant isolates are present at the same time in the same site of gastric mucosa or as interdistrict when multiple strains colonize different areas of the stomach.[21,58–60] For this reason,

Table 1 Sensitivity testing and MIC values distribution with E-test method for 61 *Helicobacter pylori* strains isolated in 50 pluri-treated pangastritis patients who underwent two to nine therapy cycles (Mascellino, unpublished data, 2013)

Antimicrobial agents	MIC values							MIC break points		Resistant strains, N (%)	Susceptible strains, N (%)
	≤0.12	≤0.25	≤1	≤8	8–32	64–128	≥256	S≤	R>		
MZ	0	0	5	6	33	14	3	8	8	50 (82)	11 (18)
CLA	4	24	8	12	7	6	0	0.25	0.5	33 (54)	28 (46)
LEV	8	32	6	10	4	1	0	1	1	15 (25)	46 (75)
TE	25	22	10	3	1	0	0	1	1	4 (7)	57 (93)
AMX	58	2	1	0	0	0	0	0.12	0.12	3 (5)	58 (95)

Notes: Total 61 strains. The antibiotic breakpoints are calculated following EUCAST 2016.

Abbreviations: AMX, amoxicillin; CLA, clarithromycin; EUCAST, The European Committee on Antimicrobial Susceptibility Testing; LEV, levofloxacin; MIC, minimum inhibitory concentration; MZ, metronidazole; R, resistant; S, susceptible; TE, tetracycline.

data regarding HR indicate that no single biopsy site can be considered representative of antimicrobial susceptibility testing and that this peculiarity can lead to underestimation of antimicrobial resistance as its detection is more difficult when *Hp* is not uniformly distributed in the stomach.[60] It should be considered that in patients with pangastritis, biopsies for *Hp* culture and susceptibility testing should be obtained from all the gastric regions, in order to obtain a full view of the inflammation status and to verify the presence of HR to antibiotics.[57]

Several articles report the phenomenon of HR in *Hp* infection. Ben Mansour et al[61] have studied this issue on a large scale distinguishing between multiple infections with genetically different isolates based on genotyping of virulence genes (*cagA* and *vacA*) and mixed infections where both antibiotic-susceptible and -resistant isolates belonged to the same genotype detected by a random amplified polymorphism DNA (RAPD profile). It has been reported that in mixed infections a resistant clone emerges from the susceptible one under selective pressure because of antibiotic consumption, whereas in multiple infections the individual is infected by two genetically distinct strains. In the first case, discordant results of susceptibility testing were detected in two biopsies from the same patient. The same authors studied *Hp* infection in populations in two different geographical areas: in Tunis with a prevalence of *Hp* infections of about 50% and in Poitiers in France with a prevalence of 22%.[61] It has been reported that multiple infections are more frequent in developing areas such as Tunis, whereas mixed infections are more frequently detected in developed countries, probably due to increased consumption of antibiotics in this area.[62] Multiple infections facilitate interstrain gene transfer and maintenance of genetic diversity. Instead, in mixed infections (susceptible and resistant strains at the same time), the resistant genes such as *rdxA* and *frxA* for MZ, 23S rRNA for CLA, and *gyrA* for LEV are not detected by RAPD-polymerase chain reaction (PCR). Thereby, both susceptible and resistant strains may express an identical genetic profile mainly in patients with single infection (unique RAPD fingerprint).[63] Mixed infections may represent a major challenge, because when only few resistant strains are present in a population of susceptible isolates, the resistant bacteria cultures are difficult to be carried out especially by phenotypic tests.[64,65] This kind of problem emerges more frequently for MZ, probably due to a higher prevalence of MZ resistance.

Kim et al[66] examined 220 pairs of isolates obtained from both antrum and corpus and found that 41 out of 109 patients (38%) showed HR in two biopsy sites and that 34% with resistant strains would be misclassified as susceptible if a biopsy of the antrum alone was used for antimicrobial susceptibility testing. It is suggested that the development of resistance is most likely caused by genomic alterations from pre-existing susceptible *Hp* rather than by a coinfection with a different strain. Furthermore, there is a possibility that genomic DNA from a resistant *Hp* can transform a susceptible strain into a resistant one.

de la Obra et al[21] found cultures containing mixed, MZ-susceptible and MZ-resistant isolates in 10% of cases. Considering the genetic relationship of the isolates showing HR, it can be highlighted that MZ resistance can be due to ex novo mutations (acquired resistance) and not to the horizontal transfer of genes among unrelated strains.

The results of our study, as reported in Table 2, showed that in the patients with HR, each pair of isolates in different gastric regions (antrum and corpus) belonged to the same genotype (cagA + s1m2 in one patient and cagA + s1m1 in three patients).[67] Yet, other authors share the opinion that an individual may have a mixed *Hp* infection, as demonstrated by the presence of different antimicrobial susceptibility in various gastric regions contemporarily harboring the same genotype.[68]

Norazah et al[69] showed that some isolates with similar PCR-RAPD differed in their antibiotic profiles because of MZ resistance in one of the stomach sites. A large degree of genetic heterogeneity was observed in *H. pylori* strains circulating among Malaysian patients. The phenomenon of HR predominantly involves MZ and CLA.

Table 2 Heteroresistance of *Helicobacter pylori* isolates to CLA, MZ, and AMX in four patients, in different districts of the stomach

Antibiotics	Patient 1	Patient 2	Patient 3	Patient 4
CLA	S (C) ➡ R (A)	S (C) ➡ R (A)	S (C) ➡ R (A)	S (C) ➡ R (A)
Both pairs in A–C Genotype	cagA + s1m2	cagA + s1m1	cagA + s1m1	cagA + s1m1
MZ			S (A) ➡ R (C)	
Both pairs in A–C Genotype			cagA + s1m1	
AMX		S (C) ➡ R (A)		
Both pairs in A–C Genotype		cagA + s1m1		

Notes: The strain genotypes, detected on the basis of virulence genes (cagA and vacA) in both pairs of isolates from different parts (antrum and corpus) in a single stomach, are the same in each site (cagA + s1m2 in one patient and cagA + s1m1 in three patients). The genotype cagA + s1m1 results to be more virulent than the genotype cagA + s1m2.[67]

Abbreviations: A, antrum; AMX, amoxicillin; C, corpus; CLA, clarithromycin; MZ, metronidazole; R, resistant; S, susceptible.

The resistance studied through a real-time PCR with hybridization probes has been mainly focused on CLA and TE.[65,70] Conventional antimicrobial susceptibility testing (E-test) fails in ~10% of attempts because of the overgrowth of contaminating bacteria or to the lack of live microorganisms. As previously demonstrated for CLA, molecular techniques such as a real-time PCR can be used as a diagnostic rescue method.[65] Thus, this assay proved to be reliable and quite sensitive in diagnosing resistant bacteria. As a matter of fact, some differences in the detection of antibiotic resistance may be observed in several strains when comparing E-test with PCR, especially if there are only few resistant bacteria among many susceptible ones. In this condition, the resistant bacteria are difficult to detect. Through phenotypic testing (E-test), the susceptible bacteria are identified first leading to a possible misclassification, whereas with real-time PCR a mixed infection with both resistant and susceptible strains is easily detected. In our study, 44 patients (71%) out of 62 with *Hp* infection were found to yield both wild types and strains carrying the mutation A21444G for CLA. Among these 44 individuals, 34 (77.2%) showed susceptibility and only 10 (28%) resulted to be resistant by E-test, whereas all 44 strains showed resistance to CLA by PCR.[57]

However, we should consider some issues because of the use of molecular methods. First of all it is necessary to highlight that the *Hp* culture with antibiotic susceptibility test remains the gold standard in the detection of this microorganism as well as of most of the bacteria in clinical practice.[71] Thus, the importance of *H. pylori* culture remains unaltered both in epidemiological and pharmacological fields. Moreover, the culture is crucial in the identification of new drugs for *Hp* in different countries as well as in the preparation for a future vaccine. Rather the problem concerns the difficulty of *Hp* growth that is slow and time consuming and not available for all routine diagnostic laboratories.

Other shortcomings of the PCR assay might be its low specificity for *Hp* or the presence of false negative even though these problems seem to be overcome by the updated methodologies.[65,70] In fact, on one hand, Oleastro et al[65] state that their assay to test CLA resistance compared to the real-time PCR assays previously reported in the literature proved to be able to detect all the common mutations associated with CLA resistance, whereas the assay described in 2001 by Chisholm et al[72] appeared to be less sensible (four biopsies out of 56 were found to be falsely *Hp* negative).

On the other hand, Glocker et al[70] show that, although the PCR primers applied for the detection of TE resistance are not specific for *Hp*, melting curve analysis allows discrimination between *H. pylori* and other *Helicobacter* species and recommend in order to exclude false positive, that all samples be tested by an *Hp* specific PCR assay (ie one that amplifies the *vacA* or *ureC* gene).

All in all, we can say that the real-time PCR should be used in case of growth failure, contamination, and mixed infections, thus obviating the need of live bacteria other than on gastric biopsy samples. In the latter case it would be possible to detect directly from the biopsy the CLA genotypic resistance that could predict possible therapy failures or cause changes in the previous treatments.[70,73] The genotypic resistance determined using stomach specimens correlates well with the genotypic and phenotypic resistance determined in *Hp* strains.[73] More importantly, this molecular assay can be conducted using stool samples without performing endoscopy.[74] In these situations PCR proves to be superior to bacterial culture.

To the best of our knowledge, there are not sufficient data in the literature in support of the fact that HR may, on large scale in clinical practice, affect *Hp* eradication outcomes. The current guidelines for *Hp* treatment do not issue any statements in this regard.

Value of culture and antibiotic susceptibility testing in the eradication rate

The resistance of *Hp* to antibiotics has become more prevalent over time so that culture-guided therapies result in a significantly lower risk of treatment failure compared with empiric standard triple therapy. Antimicrobial susceptibility testing has, therefore, been proposed as a logical first step in treatment failure (the goal in fact is to cure the infection after having obtained the result of antibiotic susceptibility test) but controlled trials suggested that it may not always be essential for clinical management.[75,76]

The question whether susceptibility testing is essential in guiding therapeutic strategies has been debated in many studies. Some data show that successful eradication can be achieved in almost all patients without susceptibility testing, whereas the results of other studies demonstrate that even a first-line therapy should rather be scheduled on the basis of sensibility/resistance of *Hp* to antibiotics.[73,77–79] Making comments on susceptibility-based therapies, Graham highlights that they will always be superior to empiric therapies in any population with the prevalence of antimicrobial resistance $>0\%$.[78] The problem of antibiotic susceptibility has mainly economic implications. Susceptibility testing is expensive and time consuming, not available in many

hospitals and being mostly reserved to a limited number of selected patients attending dedicated centers. Furthermore, it would not be possible to perform susceptibility tests in many patients who refuse to undergo endoscopy for its invasiveness and costs.

This issue has been studied in our Institution. From 2011 through 2013, we evaluated 100 patients with previous two or more therapeutic treatments, all positive on C^{13} urea breath test (UBT) and histological examination.[57] Sixty-two out of 100 subjects showed positive culture and were treated according to antibiotic-susceptibility tests generally with the standard triple therapy containing lansoprazole + AMX and either MZ or CLA (LAC or LAM). The remaining 38 patients showed no microorganism growth and thus underwent tailored empiric therapy using antibiotics not used before and the time of cure was prolonged. We observed that patients treated empirically showed a degree of eradication of 84% and the patients with positive cultures and susceptibility-based treatment, of 77%. This can be due to the fact that bacteria unable to grow on culture media were probably fewer (too low in number to be cultured) and in a less virulent or dormant phase compared to the patients where *Hp* was isolated.[57]

Many variables are involved in this issue and for this reason no uniform and homogeneous data can be found in the literature. In fact, on one hand, culture-guided therapy is essential in order to establish a correct and efficacious treatment, and on the other hand it is also reported that empiric therapy based on regional antibiotic resistance patterns achieves high eradication rates.[78–81] It would be possible to predict the efficacy of any treatment knowing the prevalence of antibiotic resistance for a regimen or even for a specific patient: as a matter of fact empiric therapy that takes into consideration the regional and mostly the local resistance patterns may be superior to predict the efficacy of any regimen.[23,80] Hence, the regional resistance patterns and the eradication rates in the context of local environment are crucial for a correct establishment of *Hp* cure in real-world settings.

Previous regimens and updated treatment strategies

Previously empirically tailored triple therapy was suggested with the association of two antibiotics: AMX (1 g) due to its low rate of resistance and either MZ (250 mg) or CLA (250 mg) given b.i.d. for 10 days combined with PPI (proton pump inhibitor 40 mg) considering the gastritis pattern.[82,83] In patients with MZ-resistant strains, this drug was administered at a higher dosage of 500 mg b.i.d. Indeed, there is evidence that the increase in MZ dosage generally improves the results of therapy when treating MZ-resistant *Hp* strains. It is confirmed that the proportion of eradication rate is significantly lower in resistant compared to susceptible strains especially with triple therapy. The eradication success rate with quadruple therapy including MZ appears to be lower in MZ-resistant versus MZ-sensitive strains (92% versus 80%), p=0.06.[84,85]

The knowledge of the local antibiotic resistance is crucial in order to establish an appropriate antibiotic therapy. It is reported that the increasing prevalence of CLA resistance is the main factor contributing to *Hp* treatment failure indicating that in the regions with CLA resistance >15%, a regimen including CLA should not be used.[84,86,87] The old triple therapy (PPI + AMX and either CLA or MZ) should be considered only in areas where resistance to CLA is low (<15%) or where a high eradication success rate with these regimens (>85%) is well known.[84] In general, in Western countries, CLA-containing triple and sequential therapies should be considered obsolete as empiric treatment.[83] Sequential therapy has been first introduced by Zullo et al and consists of 10-day therapy comprising 5 days of PPI + AMX followed by other 5 days with triple treatment containing PPI + CLA + MZ.[88] This regimen uses AMX first as it is able to overcome CLA resistance by destroying *Hp* cell walls and preventing activation of efflux channels, which is one of the mechanisms for CLA resistance. Unfortunately, this kind of therapy decreases patients' compliance.

Concomitant therapy includes PPI, CLA, AMX, and MZ for at least 10 days. This regimen has shown a better outcome over standard triple therapy especially in cases of CLA resistance. The advantages of concomitant therapy are the efficacy against dual antibiotic-resistant strains and a higher compliance rate compared with the sequential therapy.[89,90] Anyway the efficacy of concomitant therapy depends on the prevalence of *Hp* antimicrobial resistance, which varies in geographical areas. The updated guidelines for *Hp* treatment recommend to prolong therapy from 10 to 14 days. For the first-line treatment, a concomitant non-bismuth quadruple therapy (PPI + AMX + MZ + CLA, PAMC) or traditional bismuth quadruple therapy (PPI + bismuth + MZ + TE, PBMT) is recommended. Bismuth quadruple therapy is unaffected by CLA resistance.[84] In a recent survey conducted in Italy in 2016, Tursi et al[91] described the first Italian experience in clinical practice with this new bismuth-containing quadruple therapy in patients infected with *Hp*. Both naïve and previously treated dyspeptic patients were enrolled. This new therapy seems to be a more reliable option.

Patients were treated with a new formulation of drugs that uses a single pill (three in one capsules containing bismuth subcitrate potassium 140 mg, MZ 125 mg, and TE 125 mg). Three capsules q.i.d. plus omeoprazole 20 mg or esomeprazole 40 mg b.i.d. are given for 10 days.[91] *Hp* eradication rate assessed with UBT resulted to be very high: 94.7% in intention-to-treat (ITT) analysis, whereas in the per protocol (PP) population this percentage was even higher (97.6%). No difference was seen in the group studied by Tursi et al[91] between naïve population and previously treated patients. This new bismuth-containing quadruple therapy results to be very effective in both situations: as the first-line treatment and as rescue therapy. However, in this survey, some limitations may be present considering the small number of individuals enrolled and the fact that this therapy has become available in Italy only in 2016. No antibiotic tests were performed in the population under study. Nevertheless, the eradication rates were very high.[91]

Likewise, recently, Delchier et al[91] found that this new regimen used as a rescue therapy reached an eradication rate in ITT and PP populations, of 93% and 95%, respectively, showing comparable results with those obtained by Tursi.[92] All current guidelines advice to prescribe bismuth-based regimens in areas where resistance to CLA is high.

Beyond the quadruple therapy, recommended rescue therapies also include treatments based on the use of LEV (PPI + AMX + LEV, PAL). An appropriate second-line regimen should contain a quinolone; however, there is little research to support this, and it should be noted that many regions have high quinolone resistance rates.[93] The use of rifabutin (RIB) (usually PPI + AMX + RIB, PAR) should be limited to patients who failed at least three previous regimens as reported by Fallone et al[84] (Table 3). Moreover, the Toronto guidelines discourage the addition of probiotics for the reduction of side effects of the therapy or for increasing the eradication rates because the evidence in support of this thesis is very limited.[84] The Toronto Consensus Group for the treatment of *Hp* infection concluded that the treatment choice must be based on the susceptibility tests but above all, on the prevalence of antibiotic resistance as well as on eradication patterns of specific therapies in the local population. When this information is not available, the empiric alternative for adults includes quadruple therapies PAMC or PBMT for 14 days.[85] The strategy is directed to various use and consumption of antibiotics in different geographical areas over time. This means that the choice of a second-line treatment depends on previous antibiotic exposure. In fact, the selection pressure exerted at a population level with a high use of antimicrobial agents will be the reason for the emergence and spread of resistance in the population and a high incidence of primary resistance.[61]

A new drug, vonoprazan (potassium-competitive acid blocker P-CAB), recently approved in Japan, has become available for *Hp* treatment even for CLA-resistant strains.

Table 3 Recommendations for regimens used for the eradication of *Helicobacter pylori*

Recommendation	Regimen	Definition
First line		
Recommended option	Bismuth quadruple (PBMT)	PPI + bismuth + metronidazole[a] + tetracycline
Recommended option	Concomitant non-bismuth quadruple (PAMC)	PPI + amoxicillin + metronidazole[a] + clarithromycin
Restricted option[b]	PPI triple (PAC, PMC, or PAM)	PPI + amoxicillin + clarithromycin
		PPI + metronidazole[a] + clarithromycin
		PPI + amoxicillin + metronidazole[a]
Not recommended	Levofloxacin triple (PAL)	PPI + amoxicillin + levofloxacin
Not recommended	Sequential non-bismuth quadruple (PA followed by PMC)	PPI + amoxicillin followed by PPI + metronidazole[a] + clarithromycin
Prior treatment failure		
Recommended option	Bismuth quadruple (PBMT)	PPI + bismuth + metronidazole[a] + tetracycline
Recommended option	Levofloxacin-containing therapy (usually PAL)	PPI + amoxicillin + levofloxacin[c]
Restricted option[d]	Rifabutin-containing therapy (usually PAR)	PPI + amoxicillin + rifabutin
Not recommended	Sequential non-bismuth quadruple therapy (PA followed by PMC)	PPI + amoxicillin followed by PPI + clarithromycin + metronidazole[a]
Undetermined	Concomitant non-bismuth quadruple therapy (PAMC)	PPI + amoxicillin + metronidazole[a] + clarithromycin

Notes: Reprinted from *Gastroenterology*, 151(1), Fallone et al, The Toronto consensus for treatment of Helicobacter pylori infection in adults, 51–69, Copyright (2016), with permission from Elsevier.[84] [a]Tinidazole may be substituted for metronidazole. [b]Restricted to areas with known low clarithromycin resistance (<15%) or proven high local eradication rates (>85%). [c]There is some evidence that adding bismuth to this combination may improve outcomes. [d]Restricted to cases in which at least three recommended options have failed.

Abbreviation: PPI, proton pump inhibitor.

It is used for both, primary and secondary eradication, thereby addressing the health care needs in acid-related disorders.[94]

Vonoprazan could improve eradication rates by raising the intragastric pH and thus increasing bacterial antibiotic susceptibility. Recent studies revealed that P-CAB-based triple therapy was more effective than PPI-based triple therapy (76.1% versus 40.2%) as a first-line *Hp* eradication method.[95–97] Furthermore, even in the presence of CLA-resistant strains, P-CAB-based triple therapy showed good eradication rates.[87]

Conclusions and future perspectives

Hp antibiotic resistance has been increasing all over the world in the last decade and this phenomenon constitutes an important challenge for the treatment of this fastidious bacterium. Low bacterial growth, presence of coccoid forms in distinct gastric regions, extremely high resistance rates to various antibiotics in local population groups, their inappropriate associations, low dosages, or insufficient administration time may explain poor therapeutic outcomes. Whether HR might affect *Hp* eradication requires further studies.

Antibiotics once considered a first choice (such as MZ and CLA) and included in all therapeutic regimens are declining in efficacy because of their extensive use in many areas for other infections (eg, MZ in amoebic, gynecological, and anaerobic infections and CLA in respiratory diseases).

This has led to an obstinate search for new solutions such as the vaccine development and new treatments based on the use of natural resources such as plants, probiotics, and nutraceuticals.[98,99]

The search for new antibiotics has a poor therapeutic future. Other solutions should be pursued in order to increase the cure rate of *Hp* infection. Nontraditional therapies have been indicated as a means to target this important gastric pathogen. This approach includes the use of antimicrobial peptides (core component of innate immune system of numerous eukaryotes) that interact with the anionic Gram-negative cell wall because of charge electrostatic attractions.[100] It also seems reasonable to investigate other options aimed at reinforcing the immune system of these patients, in order to improve the success rates of *Hp* infection treatment. In addition, novel alternatives based on microorganisms, polysaccharides, and intragastric violet light irradiation have been proposed.[98]

The problem of the utility of sensitivity tests is still debated. Though undoubtedly useful, sensitivity tests can be a challenge, because to be carried out, they need patients' endoscopy, they are expensive, time consuming, and can only be performed in few well-equipped laboratories. The prevalence of antibiotic resistance for a specific regimen or a patient and local data on antibiotic resistance as well as eradication patterns have proved to be crucial in the management of *Hp* infections.

The Toronto Consensus suggests to prolong eradication therapy up to 14 days replacing the old triple therapy with the quadruple therapy based on PPI, bismuth, MZ, and TE (PBMT) for most of the patients and alternatively the variant quadruple therapy without bismuth: PPI, AMX, MZ, and CLA (PAMC).

The new drug vonoprazan has been associated with satisfactory eradication rates even in CLA-resistant strains.

Disclosure

The authors report no conflicts of interest in this work.

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