Chapter

Virulence Markers, Genotypic versus Phenotypic Resistance and New Treatment Strategies in *Helicobacter pylori* Infection

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Abstract

This chapter aims at studying the microbial virulence determinants and markers of *Helicobacter pylori (Hp*), the molecular diagnostic of *Hp*, the growing antibiotic resistance with the related problem of heteroresistance, the genotypic resistance to antimicrobials compared with the phenotypic methods and the new treatment strategies for *Hp* eradication also evaluating new antimicrobial agents (furazolidone, vonoprazan). The virulence markers cover an important area in *Hp* pathology due to the correlation between these and the different diseases. The *Hp* molecuar diagnosis is fast, accurate and reliable over the traditional methods that are expensive and time- consuming. Therapy regimens used over the past decade are declining in efficacy being the *Hp* treatment bedevilled by drug-resistant strains. New treatment strategies are under study worldwide. The determination of the genetic resistance to antibiotics is very useful when used directly on gastric biopsies for prediction of antibiotics ineffectiveness or for addressing changes in previous treatments.

Keywords: *Helicobacter pylori* infection, virulence markers, molecular diagnostic, heteroresistance, antibiotic resistance, updated treatment strategies

1. Introduction

Helicobacter pylori (Hp) was first isolated in culture media by Warren and Marshall in 1983. Since then much progress has been made regarding all the characteristics, the pathology and the resistance to antibiotics of this microorganism, However the history of this bacterium goes back a long time ago. In 1892 Bizzozzero described the presence of helical microorganisms in gastric mucosa of dogs and cats. In 1896 Salomon demonstrated the transmission to rats. In 1899 Iaworski & Krientis evidenced helicoidal microorganisms in human gastric biopsies. In 1967 Luck, in 1975 Steer, in 1979 Fung and in 1982 Gregory showed the bacterial ultrastructural morphology. In 1983 Marshall & Warren identified for the first time *Hp* [1]. In 1984 Langenberg hypothesized a relationship between stomach urease and spiral germs [2].

Hp is a Gram-negative, spiral-shaped bacterium, with positive findings for urease, oxidase and catalase. It colonizes the human gastric epithelium. The main pathologies related to *Hp* infection are the following: chronic active gastritis, peptic ulcer disease, gastric carcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma. Further, epidemiological and eradication studies have demonstrated a casual relationship between *Hp* infections and endothelial dysfunction, leading to vascular diseases [3, 4]. Generally, the colonization occurs primarily during childhood especially in the developing areas, usually in the same family for a cohort effect [5]. This colonization is widely asymptomatic even if a long-lasting infection can be established in some subjects. After colonization all patients with *H. pylori* infection develop histological gastritis, which corresponds to classical chronic gastritis and is characterized by the infiltration of neutrophils and other inflammatory cells. However, most patients are asymptomatic for life, while few of them will develop a digestive disease. Infection is virtually lifelong in the absence of treatment, implying that evasion of the host response is efficient.

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 the USA, Canada, Japan, and Western Europe, the prevalence is much lower (about 25–30%) [6–9].

The infection outcome mainly depends on three factors: strain virulence, host response and environmental factors. The strain pathogenicity depends on the virulence markers present in the bacterium. Host response shows the peculiarity that it is not protective, indeed in some cases may worsen the patients situation. Environmental factor, such as cigarette smoking is a major risk factor for duodenal ulceration among *Hp*-infected persons. Other important factors include stress, childhood living conditions, diet, alcohol and NSAIDs (non- steroidal anti-inflammatory drugs) use [10, 11].

Aim of this chapter was to study the virulence markers involved in the pathogenicity of *Hp* such as Vacuolating cytotoxin (VacA) and vacA gene (vacuolating cytotoxin gene A), intermediate region (i) of vacA, CagA protein and the cag pathogenicity island. The molecular diagnostic of the microorganism, the genotypic resistance related to the phenotypic one, the antibiotic resistance and the updated treatment strategies including also non-antibiotics therapy are similarly studied.

2. Pathogenicity and virulence markers

2.1 Pathogenicity

The pathogenicity of *Helicobacter pylori* is shown in **Figure 1**.

Here it is reported the course of *Hp* infection in the patients beginning from the childhood to the advanced age considering what may happen at high level or at low level of acidity. The infection starts with the colonization of the microorganism in the normal gastric mucosa which can lead to an acute *Hp* infection at a low level of acid production. This in turn can result in a chronic *Hp* infection which may be asymptomatic for a lifetime or produce a non- atrophic pangastritis which can lead to MALT lymphoma. At high level of acidity the infection can result in an antral predominant gastritis with an evolution to duodenal ulcer. At a low level of acidity it can result in a corpus predominant atrophic gastritis which may evolve in gastric ulcer, intestinal metaplasia, dysplasia and gastric cancer in 2% of infected patients [12].

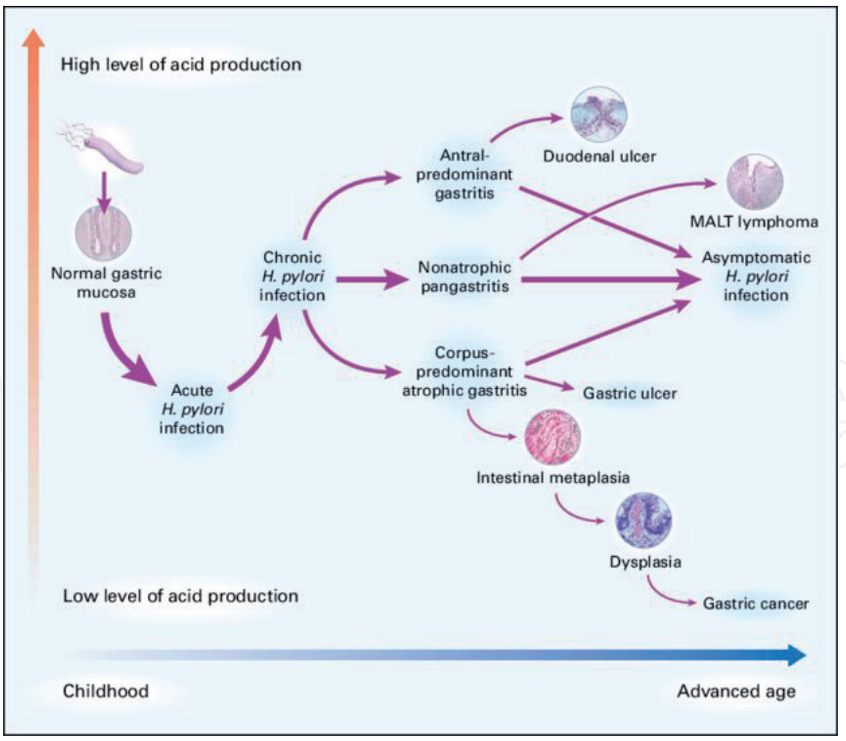

Figure 1.
Pathogenicity of Helicobacter pylori. *From Suerbaum et al. [11].*

2.2 Markers of virulence

Gastric colonization is a prerequisite for *Hp*-associated disease and this is mediated by both flagella and urease: mutant strains lacking these features cannot establish infection. The most important determinants of virulence are the following: (a) Vacuolating cytotoxin (VacA) and vacA gene (vacuolating cytotoxin gene A), (b) Intermediate region (i) of vacA, (c) CagA protein and the cag pathogenicity island, (d) Hps60 superficial protein, (e) BabA adhesion, (f) Urease virulence determinant.

2.2.1 Vacuolating cytotoxin (VacA) and vacA gene

This is a protein found in culture supernatant that induces vacuolation in a variety of cultured epithelial cell lines, The expression of *Hp* vacA gene leads to the production of a vacuolating cytotoxic protein VacA, (present only in about 40% of isolates), which is responsible for inducing the formation of acidic vacuoles. This secreted protein toxin is responsible for the gastric epithelial erosion observed in infected hosts [13].

In **Figure 2** the schematic structure of Vac A is reported [10].

In this figure the mosaicism of VacA is underlined [13]. In fact the vacA gene contains two variables regions: the s- (signal) region encoding part of the signal peptide with the N-terminus of the mature protein (hydrophilic part) and the m- (middle) region encoding C-terminal portion of the final processed polypeptide (hydrophobic part). These regions are both cleaved upon secretion to yield a mature toxin monomer of 87–95 kilodaltons [14]. The combination between the *s* and *m* regions causes the strains virulence and is correlated with the kind of disease. In fact s1-type strains are associated with vacuolating activity, s2-type is non-vacuolating, m-region causes the specificty of cell vacuolating: m1 alleles are more toxigenic than strains with m2 alleles, vacA s1m1 is the most toxigenic combination and is associated with duodenal and gastric ulceration.

The subtype s1a m1 is the most virulent strain involved in patients with ulcer.

A further region of the vacA gene (i- intermediate region) has been reported in literature to be associated with gastric cancer [15].

2.2.2 CagA protein and the cag pathogenicity island

Cag pathogenicity island is a chromosomal region with about 37,000 bp and 29 genes. Four genes are similar to the components of the type IV secretion system. Proteins encoded by the island are involved in two major processes: the induction of interleukin-8 production by gastric epithelial cells and the translocation of CagA

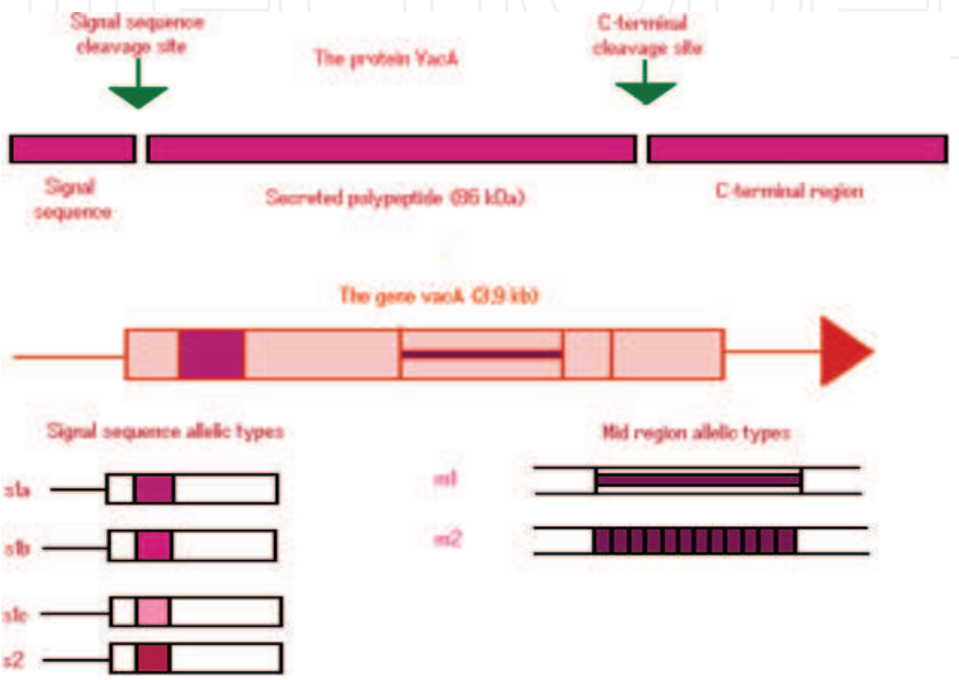

Figure 2.
The protein VacA. From Atherton et al [10].

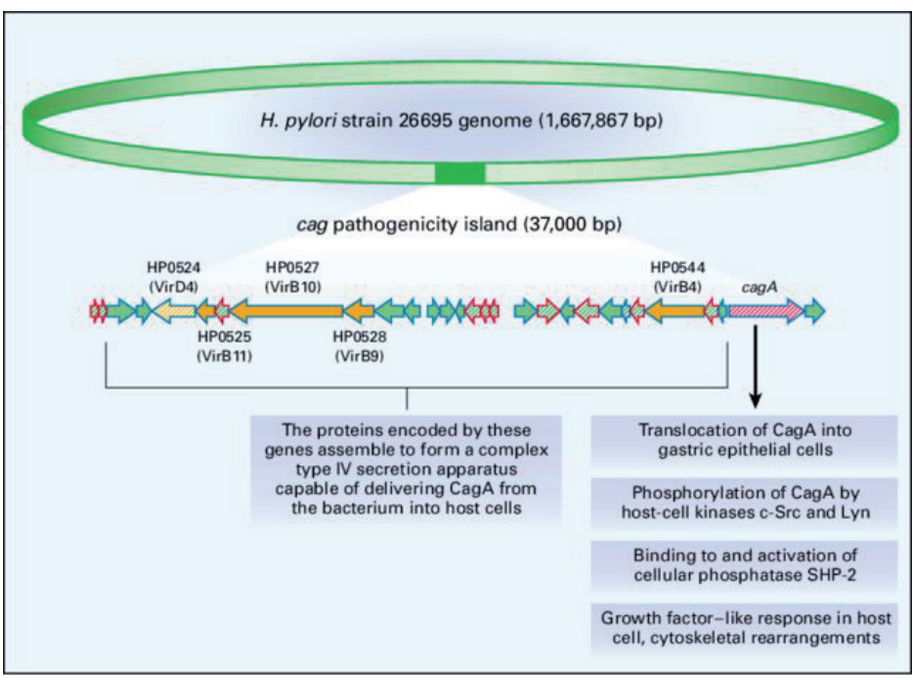

Figure 3.
The Cag pathogenicity Island. Modified by Suerbaum et al [11].

from the bacterium into the host cell by forming a syringe and a needle apparatus that delivers CagA protein (a protein of about 1200 amino-acids whose size varies between strains). Some genes are essential for the induction of interleukin-8; other genes are required for the translocation of CagA. The cagA-positive strain increases the risk of athrophic gastritis development and mucosal inflammation [16]. (see **Figure 3**).

2.2.3 Heat shock proteins (Hsps) or stress proteins

Hsps are families of highly conserved proteins serving as a strong antigenic target for the immune response linked with pathology. *H. pylori* produces 2 Hsps: a gro Es-like HspsA (size 13 kd) and a gro E1-like HspsB (size 54–60 kd). Hsp60 is shared by *H.pylori* and eukariotic cells (autoimmune response) [17, 18]. The role of heat-shock proteins in immune reactions is complex especially for the cellular effects of this proteins during the recognition processes by innate immunity. Heat-shock proteins (HSPs) are expressed at high levels by bacterial pathogens during adaptation to intracellular survival. The Hsps of both pathogens and hosts are involved in the activation of the receptors in innate immune response and in the presentation of antigens for the adaptive immune response [17, 18].

2.2.4 Bab A adhesions

The microorganism needs of adhesins for beginning its infective process. The presence of bacterial adhesins devoted to the attachment to human gastric epithelium, is essential [19]. *Helicobacter pylori* adherence to the human gastric mucosa involves specific bacterial adhesins and cognate host receptors. *babA* gene codes for the blood group antigen-binding adhesin BabA whereas the babB product is associated with a non-binding phenotype. BabA major adhesin is directed to the fucosylated Lewis b blood group antigen not present in all kinds of gastric cells [19].

2.2.5 Urease virulence determinants

The enzymatic activity of urease manages to break the urea molecule in bicarbonate ion and ammonia so that it is able to neutralize the gastric acid and is also correlated with the dual function of adhesivity and immunogenicity. The urease binds to the CD74 receptors of gastric epithelial cells [20]. It can be suggested that urease is specifically important for the attachment of *Hp* and for the pro-inflammatory immune response initiated by the bacterium. The binding of the subunit urease B to CD74 expressed on gastric cells may therefore contribute to increase the bacterial virulence during infection [20].

3. Molecular diagnostic of *Helicobacter pylori*

Helicobacter pylori is a fastidious bacterium difficult to grow in the common media culture. Essential conditions for *Hp* culture were the following: microaerophilic atmosphere, temperature of 37° (range 33°-40°), presence of 0.5% glycine. The appropriate culture medium such as Pylori Selective Agar (bio-Merieux, Marcy L'Etoile France) contains 5% sheep blood and antibiotics (amphotericin, vancomycin and trimethoprim). The incubation lasts 10 days under CO_2 atmosphere. This method is complicated, expensive and time-consuming so that when the isolation of the strain is unnecessary, the molecular method is very useful for its rapidity and appropriateness.

Helicobacter pylori presence in gastric specimens can be assessed by various methods including molecular PCR assay (GenoType HelicoDR kit) [21]. PCR is a more sensitive method (84.3% sensitivity, 75.0% specificity), with a higher *H. pylori* detection rate compared to culture [22].

Tissue obtained from gastroscopic biopsy was minced using a sterile scalpel, lysed by tissue lysis buffer and proteinase-K enzyme (Bioneer, Daejeon, Korea), and incubated for 10 min at 60°C. Ten total DNA was extracted with an AccuPrep Genomic DNA Extraction Kit (Bioneer, Daejeon, Korea). This kit contains a glass filter in a column tube that can bind efficiently to DNA in the presence of salts. Additional washing steps were performed for proteins and salt removal. Aliquots of 50 μL were used for PCR amplification as reported elsewhere [23].

Molecular methods such as PCR offer marginal improvements when done on biopsy material, but have the advantage of being able to accurately identify *H. pylori* in areas outside the stomach where cultures usually fail. PCR can detect low numbers of organisms in gastric juice, bile, stool and oral secretions. Because of its high sensitivity it can also be used for epidemiologic investigations of environmental sources [24]. Molecular methods have been used in variable specimens other than gastric mucosa [25].

4. Antibiotic resistance and heteroresistance

4.1 Antibiotic resistance

The antibiotics once regarded as the first choice for *Hp* infection (such as metronidazole and clarithromycin), included in all therapeutic regimens, are now declining in efficacy because of their extensive use in many areas for unrelated infections. Metronidazole (MZ) mostly showed a very high resistance worldwide achieving a level up to 78.2% in China [26]. Clarithromycin (CLA) resistance rates have currently reached high levels, such as 30% in Italy and Japan, 40% in Turkey, and more than 70% in China, although rates in Sweden, Taiwan, and Germany are lower [27]. CLA was once considered the most powerful antibiotic for *Hp* infection. The local pattern of *Hp* resistance to CLA results as being crucial in each area, considering 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. Fluoroquinolone (the most common fluoroquinolone is Levofloxacin LEV) resistance has been increasing worldwide in recent years achieving 20% in Italy, 13.3% in Germany, and 19.2% in China [26, 28]. These data are especially important in those regions planning a levofloxacin-based therapy because resistance to fluoroquinolones generally shows a major impact on the success of treatment [29].

In a study conducted in an Academic Hospital in Rome by our group on 80 *Hp* strains isolated from 80 biopsy samples in infected patients, the resistance percentage measured phenotypically through MIC values to MZ, CLA, LEV, TE (tetracycline) and AMX (amoxicillin) resulted as follows: 61.6% (50/80), 35% (28/80), 20% (16/80), 2.5% (2/80), and 1.25% (1/80), respectively. The MICs (Minimum Inhibitory Concentrations) resulted as being very high mainly to MZ showing three strains with MIC = 256 μg/mL and to CLA showing four strains with MIC ranging from 64 μg/mL to 128 μg/mL. As for LEV the MIC ranged from 0.25 μg/mL to 32 μg/mL (only one strain showed a MIC equal to 32 μg/mL) [30].

Multidrug resistance (the contemporaneous resistance to two or more antibiotics) is reported in **Figure 4**.

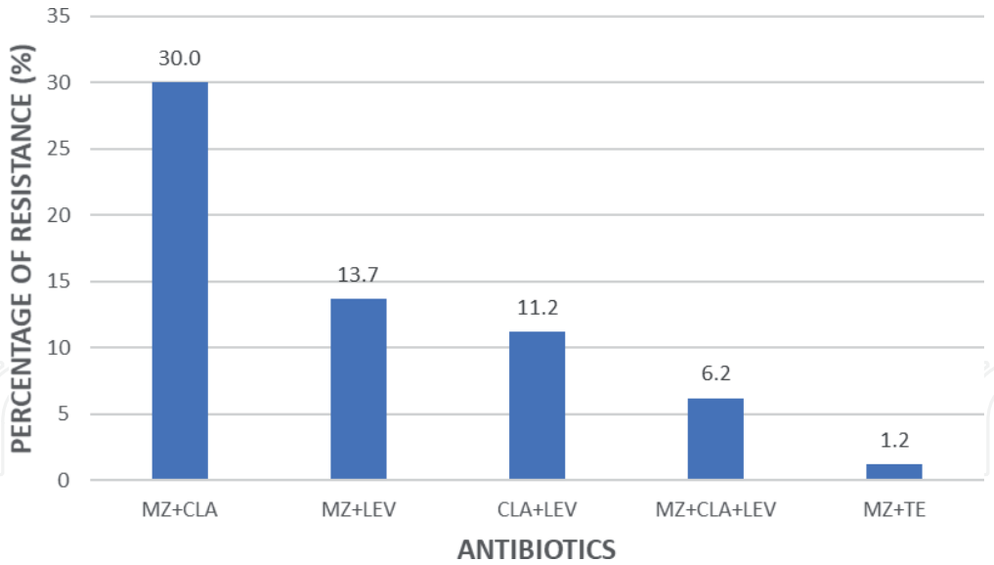

Figure 4.
Characteristics of phenotypic antibiotic resistance in Helicobacter pylori *strains. From Mascellino et al. [30]. Notes: Proportion of phenotypic resistant* Helicobacter pylori *isolates (%) to the respective antibiotics in an Academic Hospital in Rome (Italy). Abbreviations: MZ: metronidazole; CLA: clarithromycin; LEV: levofloxacin; TE: tetracycline. Resistance belonging to the association CLA + MZ was the highest one being detected in 30% of samples (24/80), resistance to the combinations MEZ + LEV and CLA + LEV ranged between 13.75% (11/80) and 11.25% (9/80 strains) respectively. Only 6.25% samples (5/80) harbored the triple resistance MZ + CLA + LEV. As far as the dual combination MZ + TE is concerned, resistance was found just in one strain (1.25%). In China too, a similar trend of multidrug resistance of* Hp *is found [26]. In general, the antibiotic combinations consisting of MET, CLA and LEV had higher combined resistance rate, whereas the antibiotic combinations recommended in bismuth-containing quadruple therapy (Bismuth, Omeoprazole, MZ, TE) had lower combined resistance rates.*

Antibiotics Patients	**MZ Strain genotype**	**CLA Strain genotype**	**AMX Strain genotype**	**LEV Strain genotype**	**TE Strain genotype**
1		S (A) R(C/F) cagA+s1m2	S (A) R(C/F) cagA+s1m2		
2	S (A) R(C/F*) cagA+s1m1	S (A) R(C/F*) cagA+s1m1			
3		S (A) R(C/F) cagA+s2m2	S (A) R(C/F) cagA+s2m2	S (A/C) R(F) cagA+s2m2	S (A) R(C/F) cagA+s2m2
4		S (A) R(C/F) cagA+s1m1		S (A) R(C/F) cagA+s1m1	
5	S(A) R(C/F) cagAs1m1	S (A) R(C/F) cagA+s1m1	S (A) R(C/F) cagA+s1m1	S (A) R(C/F) cagA+s1m1	

MZ-metronidazole, CLA-clarithromycin, AMX-amoxicillin, LEV-levofloxacin, TE-tetracycline S-susceptible, R-resistant, A-antrum, C-corpus, F-fundus.
**No growth.*

Table 1.
Heteroresistance of 5 strains (Mascellino, unpublished data).

4.2 Heteroresistance (HR)

Heteroresistance is defined as the concomitant presence of a different susceptibility pattern in the different districts of a single stomach in the same patient [31]. 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 intra-district when susceptible and resistant isolates are present at the same time in the same site of gastric mucosa or as inter-district when multiple strains colonize different areas of the stomach [32, 33]. Heteroresistance of 5 strains out of 80 considered above is reported in **Table 1**.

For each of these patients, we found a different antimicrobial pattern in the strains isolated from antrum (susceptible) and corpus/fundus (resistant) towards CLA (five patients), MZ (two patients), AMX (three patients), LEV (three patients) and TE (one patient). The strain genotypes, identified on the basis of virulence genes (cagA and vacA), were the same for both loci in pairs of isolates (susceptible and resistant) obtained from different regions (A,C and F) of a single stomach (cag + s1m2 in one patient, cagA+s1m1 in three patients and cagA s2m2 in one patient).

5. Phenotypic resistance versus genotypic resistance

The phenotypic resistance was calculated on the basis of MIC (Minimum Inhibitory Concentration) performed on the biopsy samples. Interpretation of susceptibility test results was performed in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST 2020) recommendations [34]. In order to define strains resistance the following MIC breakpoints were used: $S \leq 0.125$ μg/ml and $R > 0.125$ μg/ml for AMX; $S \leq 0.25$ μg/ml and $R > 0.5$ μg/ml respectively for CLA; $S \leq 1$ μg/ml and $R > 1$ μg/ml for both TE and LEV; $S \leq 8$ μg/ml and $R > 8$ μg/ml for MZ.

The genotypic resistance is based on the point mutations present on the chromosome. Common resistance mechanisms to clarithromycin include point mutations in the bacterial domain V of 23S rRNA, which prevents antibiotic binding [35]. There are 3 point mutations in the 23S rRNA gene: A2143G, A2142G, and A2142C; these account for 90% of cases for primary resistance in Western countries [36]. Fluoroquinolone agents (mainly levofoxacin, which is a broad-spectrum quinolone) are an alternative therapy for infections caused by *H. pylori* and serve as second-line treatment [37]. Fluoroquinolone targets are the DNA gyrase, an enzyme responsible for negative supercoiling during the DNA replication process. This enzyme contains two A subunits and two B subunits, encoded by *gyrA* and *gyrB*, respectively [38]. Resistance to clarithromycin and fuoroquinolones, which is mostly acquired through point mutations, can be detected by molecular techniques. GenoType HelicoDR (Hain Life Science, Germany) test is a molecular diagnostic method for easy and simultaneous detection of frequent point mutations responsible for clarithromycin and fuoroquinolones resistance [21].

Genotypic susceptibility testing can be also performed by a real-time PCR followed by melting curve analyses using fluorochrome labeled hybridization probes for identifying the mutations on the 16S rRNA conferring resistance to TE [28].

5.1 Correlation between genotypic and phenotypic methods

The correlation between genotypic and phenotypic methods is reported in **Table 2**.

The PCR method results as being very useful in detecting the resistant strains in comparison with the E-test technique which primarily detects the susceptible ones. Mixed *Hp* infections were demonstrated only through the PCR in patients concomitantly yielding both wild type and resistant strains in a single gastric region. The resistance with both methods (phenotypic tests and PCR molecular tests) for CLA

Clarithromycin (CLA) Resistance 21 patients/60 (35%)		**Levofloxacin (LEV) Resistance 12 patients/60 (20%)**	
PCR	**E-test**	**PCR**	**E-test**
4 Wildtype + A2143G*	3 R	6 *gyr A* mutation codon 87	5 R MIC>1mcg/ml
12 A2143G	8 R MIC>0.5 mcg/ml	4 *gyr A* mutation codon 91	
16/21 R (76%)	12/21 R (57%)	10/12 R (83%)	6/12 R (41%)

S = susceptible, R = Resistant, A2143G = point mutation on CLA23SrRNA gene conferring resistance to CLA, gyrA = gene conferring resistance to LEV.
**Refers to mixed iinfections (from Mascellino et al. [39]).*

Table 2.
Correlation between genotypic and phenotypic methods.

and TE demonstrated the superiority of PCR over the phenotypic test (data not shown). As for CLA, the resistance was detected in 76% (16/21) of patients by PCR and 57% (12/21) by E-test, whereas for LEV the same values corresponded to 83% (10/12) and 41% (6/12) respectively. In this last case the difference turned out to be statistically significant ($p < 0.05$).

The genotypic test performed directly on the biopsy samples shows some great advantages over the E-test. In fact the genotypic-resistance resulted very useful to identify mixed infections that represent a real problem possibly leading to a resistance underestimation or in the case of absence of live bacteria (coccoid forms) or contamination. The real-time PCR detects the resistant population at a lower concentration than the phenotypic tests which primarily show susceptible bacteria. Following our results [30], the E-test is unable to detect all resistant strains because when there are many susceptible bacteria compared with the resistant ones, these susceptible bacteria are identified first leading to a misclassification. On the contrary through a direct examination of gastric samples by genetic tests, a diversity of strains (susceptible, resistant or both) can be detected at the same time. Mixed *Hp* infections were demonstrated in patients yielding both wild type and resistant strains in a single gastric region at the same time. The use of genotypic tests directly on the clinical specimens could predict the antibiotic resistance addressing changes in previous treatments or for evaluating the primary resistance to antibiotics (ie CLA) in order to avoid administration of ineffective antimicrobials [23, 38].

6. Antibiotic therapy

Therapy regimens used over the past decade are declining in efficacy being the *Hp* treatment affected by drug-resistant strains. New treatment strategies are under study worldwide. The knowledge of the local susceptibility to the antibiotics in a single geographical area is crucial in order to establish a correct therapy. For instance as far as CLA susceptibility is concerned, it is stated that in those regions that show a resistance percentage > 15%, this antibiotic should not be used [30]. Indeed 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 the tailored therapy in predicting the efficacy of any regimen [40]. 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 [41].

The old triple therapy (PPI=Proton Pump Inhibitors + clarithromycin and either amoxicillin or metronidazole) should be considered only in areas where the resistance to CLA is low (<15%) or where a high eradication success with these regimens (>85%) is well known. In general, in Western countries, clarithromycin-containing triple and sequential therapy should be considered obsolete as empiric therapies.

The resistance to CLA is reported to be increasing all over the world, and in some countries, it depends on the local seropositivity rate [42]. As far as LEV is concerned, there is a more limited number of studies evaluating susceptibility to LEV. In Italy LEV resistance has been reported to be 22–24% as well as in Portugal [43]. MZ shows a high rate of resistance (50–80%) in almost all the studied countries achieving a rate of 80% especially in developing areas [26]. Nevertheless in spite of its high resistance in vitro, it is included in the BQT (Bismuth Quadruple Therapy). 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 vivo [44]. The Bismuth Quadruple Therapy (PPI + Bismuth +MZ + TE] for 14 days has proven high efficacy in spite of MZ resistance in Europe bypassing also the quinolone resistance [29].

The new guidelines for the cure of *Hp* recommend to prolong the therapy from 10 to 14 days [45]. As first line therapy a concomitant non-bismuth quadruple therapy (PPI + AMX + MZ + CLA) may be used in those countries where the resistance to CLA is <15% otherwise the traditional bismuth quadruple therapy unaffected by CLA resistance, should be used. The BQT (PPI + Bismuth+MZ + TE, PBMT) results as being very useful in the countries where particular *Hp* high resistance is detected and when the AST (Antimicrobial Susceptibility Testing) is complicated to perform. In contrast if a bismuth-based quadruple therapy is used in these different situations, it is not recommended to perform AST because a risk of having a TE-resistant strain is extremely low and it was shown that MZ-resistance has no impact in the treatment of patients [29]. Recommended rescue therapy includes LEV as a second line of treatment ie PPI, AMX, LEV (PAL) [45] these therapeutic options are reported in **Table 3**.

Recommendations	Type of therapy	Drug
First line	• Old triple therapy*	PPI + CLA + either AMXorMZ
	• Concomitant non- bismuth quadruple therapy	PPI + AMX + MZ + CLA**
	• Bismuth quadruple therapy (BQT)	PPI + bismuth+MZ*TE ***
Second line	• Concomitant non bismuth quadruple therapy.	See above
	• BQT	See above
	• LEV- containing therapy	PPI + AMX + LEV
Rescue therapy	• Rifabutin containing therapy (undetermined)	PPI + AMX + RIF
	• LEV-containing therapy	See above

Notes: The therapy should be prolonged for 14 days.
PPI = proton pump inhibitor, CLA = clarithromycin, MZ = metronidazole; TE = tetracycline; AMX = amoxicillin, RIF = rifabutin.
**Obsolete therapy.*
***Used in the countries where CLA – resistance is <15%.*
****Unaffected by CLA - resistance.*

Table 3.
Antibiotic therapy.

7. New treatment strategies

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. This has prompted the researchers 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 [46, 47].

A new compound, a guanidine derivative bearing adamantane-1-carbonyl 2-bromo-4,6-difluouro-phenyl substituents (H-BDF), seemed to be promising against the strains tested [48]. Other substances were studied against *Hp* such as three known and five unknown N-substitute-2-oxo- H-1-benzopyran-3- carboxamides (coumarin-3-carboxamides). The compounds with a 4-acyl-phenyl group showed the best activity against *H. pylori* metronidazole- resistant strains [49].

Non-traditional therapies have been indicated as a means to target this important gastric pathogen. This approach also 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 [50]. It also seems reasonable to investigate other options aimed at reinforcing the immune system of these patients, The potential role of N-acetil-cysteine which is capable of destroying bacterial biofilm, is an emerging treatment for recalcitrant infections [51]. Vonoprazan (potassium-competitive acid blocker P-CAB) is a new compound which could improve eradication rates by raising the intragastric pH and thus increasing bacterial antibiotic susceptibility [52]. 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 [53]. Furthermore, even in the presence of CLA-resistant strains, P-CAB-based triple therapy showed good eradication rates [52].

Furazolidone (FUR) is a new antibiotic studied mainly in China which has demonstrted a great activity either alone or in combination with other antibiotics against *H. pylori*. It is a monoamine oxidase inhibitor which can interact with the metabolism of tyramine causing side-effects such as vomiting, diarrhea and nervous system disorders. In China, the FUR is available for the treatment of patients. Therefore, quadruple therapy with proton pump inhibitors, bismuth and a combination of two antibiotics specifically FUR, TET or AMX would be more suitable for Chinese patients [26]. This treatment is also recommended in the Maastricht V/Florence Consensus Report [29] the new treatment strategies are shown in **Table 4**.

Compounds	**Activity**
Guanidine derivate	Promising activity against *Hp* strains
Coumarin-3-carboxamides	Activity against *Hp*-MZ--R
Antimicrobial peptides	Interaction with Gram- neg cell wall
N-acetyl-cysteine	Activity on the bacterial biofilm
Vonoprazan (potassium competitive acid blocker)	Raises the intragastric pH
New antibiotic	**Action**
Furazolidone * (monoamine oxidase inhibitor)	PPI + bismuth+FUR+ either TE or AMX

Fur = Furazolidone, MZ = Metronidazole, TE = tetracycline, AMX = amoxicillin, R = Resistant.
**Used alone or in combination with other antibiotics (in western countries).*

Table 4.
New treatment strategies and non-traditional therapies.

8. Conclusions

Helicobacter pylori is a complex microorganism that is difficult to cultivate, and treat also presenting particular characteristics of pathogenicity and virulence markers. Much progress has been made since 1983 when it was discovered first by Warren and Marshall [1] making it one of the most studied bacteria. *Hp* is well adapted to the human host as evidenced by its chronical persistence in the gastric niche and by the finding that the bacterial surface carries structures (antigens) which are identical to those found on human cells [54].

The markers of virulence such as CagA and VacA are seen to greatly influence the outcome of *Hp* infection. The combination of different virulence genotypes is the most important factor that strongly affects the bacterial virulence making some strains more pathogenic than others.

The heteroresistance is a big problem in the evaluation and management of *Hp* resistance in the infections. In fact the heteroresistance detected in five of our patients worsens the situation being an important issue as an isolate could be mistakenly considered susceptible if a single biopsy is used for antimicrobial tests possibly leading to a resistance underestimation [31].

The emergence of PCR method provides a quick, convenient way to guide tailored therapy in clinical practice of *H. pylori* [55]. In this situation without performing the susceptibility tests which are time-consuming and expensive, the gastroenterologists could establish a suitable treatment for *Hp* infected patients who in any case underwent a gastroscopy. The use of genotypic tests directly on the clinical specimens could predict the antibiotic resistance addressing changes in previous treatments. The real-time PCR detects the resistant population at a very low concentration not detectable by phenotypic tests which primarily show susceptible bacteria. The genotypic-resistance is useful in case of absence of live bacteria, contamination or for identifying mixed infections.

The CLA-resistance levels and mainly the local susceptibility turn out to be crucial to establish a correct therapy. The quadruple therapy (BQT) with proton pump inhibitor, bismuth and a combination of two antibiotics, specifically MZ and TE then unaffected by CLA and LEV resistance, turns out to be appropriate in the *Hp* infected- patients resulting in an effective eradication rate.

The lack of data on local susceptibility patterns and on eradication success rates was identified as a knowledge gap that has a major impact on the choice of therapy and hence best management. Periodic susceptibility testing should be considered by health authorities and clinicians should be encouraged to record their successes.

All in all we can say that this microorganism since its discovery in 1983 by Marshall and Warren, has been deeply studied and considered one of the most important gastric pathogen involved in a wide range of pathologies other than the classical ones, such as cancer, atherosclerosis and endothelial dysfunction leading to vascular diseases.

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Conflict of interest

The authors declare no conflict of interest.

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