

DRUG RESISTANCE IN *HELICOBACTER PYLORI*

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ABSTRACT - Background - *Helicobacter pylori* has a worldwide distribution and is associated with the pathogenesis of various diseases of the digestive system. Treatment to eradicate this microorganism involves the use of a combination of antimicrobials, such as amoxicillin, metronidazole, clarithromycin, and levofloxacin, combined with proton pump inhibitors. Although the current therapy is effective, a high rate of treatment failure has been observed, mainly because of the acquisition of point mutations, one of the major resistance mechanisms developed by *H. pylori*. This phenomenon is related to frequent and/or inappropriate use of antibiotics. **Conclusion** - This review reported an overview of the resistance to the main drugs used in the treatment of *H. pylori*, confirming the hypothesis that antibacterial resistance is a highly local phenomenon and genetic characteristics of a given population can influence which therapy is the most appropriate.

HEADINGS - *Helicobacter pylori*. Microbial drug resistance. Point mutation.

INTRODUCTION

Helicobacter pylori is a spiral, Gram-negative bacteria that is linked with the pathogenesis of several diseases of the digestive system⁽¹⁰⁷⁾. Infection caused by *H. pylori* in the gastric mucosa is associated with inflammatory processes, resulting in gastritis, peptic ulcer disease, and neoplastic processes, as well as adenocarcinoma and mucosal-associated lymphoid tissue (MALT)⁽¹⁹⁾.

Although *H. pylori* infection has a worldwide distribution, its prevalence has been decreasing in some regions^(39,60). However, the wide-scale prevention of the spread of this bacteria has not yet been achieved, mainly due to the high failure rates of the eradication therapeutics that are available that have been observed in many regions of the world. In the last 5 years, studies have highlighted variations in the rates of resistance of the main antibiotics used to eradicate *H. pylori* (Figure 1)^(7,8, 22,68,71,73,80,82,95).

The use of triple therapy to treat *H. pylori* infection, which consists of proton pump inhibitors, clarithromycin, and amoxicillin or metronidazole, has become universal; however, the most recent data indicates that this combination of treatments has lost efficacy and is now only effective in a maximum of 70% of patients. In areas of high clarithromycin resistance,

bismuth-containing quadruple treatments are recommended for first-line empiric treatment. The other alternative is a second-line treatment based on proton pump inhibitors, levofloxacin, and amoxicillin⁽⁵³⁾.

Specific SNP (single nucleotide polymorphisms) are the main molecular basis of drug resistance in *H. pylori* infections and the pressure that determines the selection of resistant strains is related to frequent and/or inappropriate use of antibiotics^(11,34,79).

In the event that second-line treatment fails, antimicrobial susceptibility testing should be performed prior to any further treatment⁽⁵³⁾. While phenotypic methods represent the gold standard for drug susceptibility tests, *H. pylori* is a fastidious microorganism that is difficult to detect through the phenotypic approach; as such, drug resistance can be determined using molecular methods⁽⁵⁵⁾. This review examines current knowledge about the drug resistance of *H. pylori*.

RESISTANCE MECHANISMS OF *H. PYLORI*

Bacteria's ability to adapt and become resistant to antibiotics has made it increasingly difficult to treat many bacterial infections. This has proven to be the case with the gastric pathogen *H. pylori*. *H. pylori* is associated with diseases such as gastritis, peptic ulcers,

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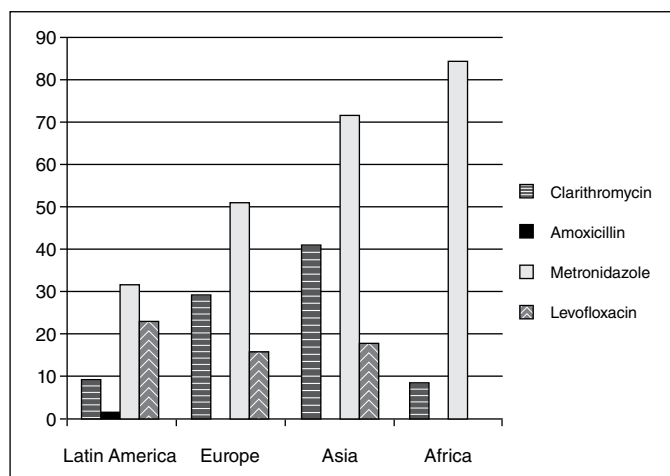


FIGURE 1. Geographic distribution of the mean between the resistance rates to the main antibiotics used to treat *H. pylori* in the last 5 years (2010-2015).

and carcinoma. The molecular basis of drug resistance in *H. pylori* has been found to result from the drug efflux mechanism or can be attributed to the presence of mutations⁽⁵⁵⁾.

Drug efflux results from the action of a transporter protein that may result from a reduction of the antimicrobial concentration inside of a bacterial cell, increasing its chances of surviving in the presence of antimicrobials. Three pumps of the RND family (*hefC*, *hefF*, *hefI*), have been shown to be involved in multidrug efflux, including clarithromycin⁽⁴⁵⁾. Furthermore, recent research has demonstrated that the overexpression of *hefA* pump may be related to the initial step in the bacteria's acquisition of resistance to the metronidazole in *H. pylori*⁽¹⁰¹⁾. However, the main *H. pylori* resistance mechanism results from the acquisition of point mutations. With regard to clarithromycin, the mutation mechanism is mainly in the 23S rRNA region, while the amoxicillin resistance acquisition is associated with mutation in the penicillin binding proteins (PBPs), and metronidazole and levofloxacin are associated with a mutation in the *rdxA* and *gyrA* genes respectively^(21,30,104,106). *H. pylori*'s resistance to the main drugs used in therapy have been frequently evaluated; however, in countries like Brazil, studies that have examined the association between the presence of point mutations and resistance to *H. pylori* are often restricted to clarithromycin.

METHODS FOR DETECTING RESISTANCE

In routine clinical microbiology, the detection of susceptibility to antibiotics is mainly based on phenotypic methods. However, *H. pylori* has slow growth and requires specific culture medium, making this phenotypic approach challenging and slow. Allied to this, due to the fact that the main antimicrobial resistance mechanism is often associated with point mutations, studies have proposed the implementation of a molecular approach from gastric biopsy specimens of *H. pylori* in clinical practice^(12,16,55,90).

Relationships between phenotypic and PCR methods have shown an excellent concordance for the detection of resistance to clarithromycin, as can be seen in Table 1^(1,2,12,26,47,49,52,72). These data show the validity of using molecular methods in clinical practice, as well as these tests detect resistance and allow the definition of the molecular basis related to it, also outweigh delays the laborious *H. pylori* culture.

TABLE 1. Comparison of the results of studies between phenotypic and genotypic method for determining resistance clarithromycin

Region	Phenotypic (n)	Genotypic (n)	Agreement between the two methods	Kappa index	Reference
Brazil	20/202	18/202	90%	0.94 *	Magalhães et al. ⁽⁵²⁾
Brazil	12/45	12/45	100%	1 *	Garcia et al. ⁽²⁷⁾
Brazil	24/114	27/114	97%	0.92 *	Lins et al. ⁽⁴⁹⁾
Spain	42/118	34/118	81%	0.84 *	Agudo et al. ⁽²⁾
France	24/229	24/229	100%	1 *	Burucoa et al. ⁽¹²⁾
France	10/59	11/59	98%	0.94 *	Lascols et al. ⁽⁴⁷⁾
Iran	32/147	31/147	97%	0.98 *	Abadi et al. ⁽¹⁾

* P<0.001

Some studies have used molecular assays, such as PCR-RFLP, to detect *H. pylori* infection and to identify the specific mutations in the 23S rRNA sequence that confer resistance to clarithromycin, which is the antibiotic of first choice in the treatment of *H. pylori*⁽⁵⁶⁾. This technique has been proven to be simple, fast and accurate to execute. As such, it has the potential to overcome the delays associated with conventional culture method and, therefore, may be useful for routine practice^(3,16,91). Data in the literature has shown that mutations in the 23S rRNA of *H. pylori* seem to be more suitable resistance markers for the purposes of predicting treatment outcomes than phenotypic analysis⁽⁵¹⁾.

The PCR-RFLP technique has also been well documented in molecular typing of *H. pylori* strains that affect subjects before and after treatment for eradication of this bacteria, which makes this fundamental detection type^(25,35). The reappearance of *H. pylori* in a previously treated patient can occur through two distinct mechanisms: recrudescence and re-infection. Recrudescence involves the reappearance of the original *H. pylori* strain after its temporary suppression, indicating that it was not effectively eradicated, while reinfection occurs when, after successful eradication, a patient is infected with a new strain of *H. pylori*⁽¹⁵⁾. It is possible to differentiate between re-infection and the recurrence of *H. pylori* strains in patients after a standard triple therapy using PCR-RFLP of a fragment of the *ureC* gene. This method is considered to be fast, effective and useful for epidemiological studies of *H. pylori* infections and for monitoring patients before and after eradication therapy^(25,35,48,59,63,87,88).

DRUGS USED IN ANTI-*H. PYLORI* THERAPY

Clarithromycin

Clarithromycin is a drug of the macrolide family that is used as an essential component of standard triple therapy for *H. pylori*⁽¹⁸⁾. However, the successful treatment of *H. pylori* in regimens containing this antibiotic is declining, and this phenomenon can be caused by prolonged use of clarithromycin to treat other infectious diseases, which favors the emergence of resistant microorganisms due to the development a selection pressure⁽⁹³⁾. The main mechanism of action of the macrolides is to inhibit protein synthesis-dependent RNA by binding to receptors located in the 50S ribosome, especially in the 23S rRNA, the region in which the main resistance mutations associated with *H. pylori* clarithromycin are located⁽¹⁰⁴⁾.

According to the increase in resistance rates over time, we can classify resistance according to three categories. "Primary", in which resistance is linked to previous exposure to the antibiotic for the treatment of other infections unrelated to *H. pylori*, such as respiratory tract infections; "secondary", resulting from a previous infection of *H. pylori*, and "tertiary" resistance, which is related to more than one episode⁽¹⁰⁸⁾.

In Europe, an increase in resistance to this macrolide has been observed. Between 2005 and 2012 in Germany, the rates of primary, secondary and tertiary resistance were recorded as 7.5%, 63.2% and 75.4%, respectively⁽⁸³⁾. While in France, the primary and secondary resistance rates were observed at 18.6% and 41.6% from 1993-1996 and 2001-2004 respectively. This increase in resistance confirms that prior treatment with clarithromycin increases the chances that an individual will acquire resistance to this antibiotic. In addition, the rates of the primary resistance have remained high in some European countries, like Poland and Italy, with rates of 24% and 35% respectively^(7,74,80).

Likewise, in Japan, a study that evaluated a total of 3,307 clinical isolates of *H. pylori* observed an increase in the levels of resistance over time, from 18.9% (2002 to 2003) to 27.7% (2004 to 2005). Another study in Japan evaluated 750 patients undergoing first-line therapy eradication, and found that primary resistance to clarithromycin increased significantly from 8.7% between 1997 and 2000 to 34.5% between 2007 and 2008. In addition, the eradication rate decreased significantly from 90.6% to 74.8%. These studies suggest the need for monitoring the resistance and development of new therapeutic regimens for the first-line eradication of *H. pylori*^(43,81). On the other hand, a large geographic variation has been observed, and an uneven distribution is possible, even within the confines of a single country, since low resistance rates have also been detected in Asia⁽¹¹²⁾. In Taiwan, with a resistance rate of 6.6%, it is indicated the use of clarithromycin as part of *H. pylori* routine treatment in this Asian population⁽¹⁰⁹⁾.

Similarly, studies have observed low resistance rates of *H. pylori* to clarithromycin in Africa and also in Latin America in countries such as Paraguay (2.2%) and Colombia (3.8%)^(3,23,82,95). However, in a meta-analysis of Latin American populations,

the summary prevalence of primary and secondary resistance was 12%⁽¹⁴⁾. In Brazil, the use of clarithromycin has been considered a good option for the treatment of first-line anti-*H. pylori*^(22,71). However, between 2000 and 2014, the rates of primary resistance ranged from 7% to 27% in Southern and South-Eastern areas of Brazil, while a rate of 16.5% was observed in the North-Eastern area (Table 2)^(22,26,28,49,52,57,71,75). These variations in resistance rates reinforced the observations of Sierra et al. (2013), who argued that the therapeutic order should be carried out in accordance with local antimicrobial resistance studies; therefore, clarithromycin should be prescribed cautiously and, when possible, after susceptible drug determination⁽⁸⁴⁾.

The increasing use of clarithromycin to treat several infectious diseases may have resulted in the development of selected resistant *H. pylori* strains and the main mutations that occur in 23S rRNA region, especially in A2143G, A2142G, and A2142C^(52,58,65,104). Additional mutations have also been observed in the V region of the 23S rRNA gene, including T2182C⁽⁴⁰⁾, G2224A⁽³²⁾, and T2215C⁽⁸⁶⁾.

In particular, the A2143G mutation has an impact on the assignment of global resistance. European countries show the A2143G mutation predominantly in strains resistant to clarithromycin, with rates of 85.0% and 90.0% in Spain and France respectively^(2,74). In Poland, Klesiewicz et al. (2014) found the same frequency of A2143G and A2142G mutation in all tested samples; however, the A2143G point mutation was correlated with a low level of phenotypic resistance to clarithromycin in comparison to the A2142G mutation⁽⁴²⁾. In Spain, the T2182C mutation, also described in the V region of the 23S rRNA gene, was detected; however, less often (5.9%) than the A2143G mutation (85.3%) among the strains resistant to clarithromycin⁽²⁾. Similarly, Asian countries, to the detect the T2182C mutation, suggest a lower frequency identification that the mutation A2143G, or yet not associated with resistance to clarithromycin, since the rate in susceptible strains to the clarithromycin is high in China (86.7%)^(40,113).

Likewise, in Latin American countries, such as Uruguay and Colombia, the A2143G mutation was the most frequent among strains resistant to clarithromycin^(3,100). Several studies have been conducted in the southeast area of Brazil to assess the point mutations in the 23S rRNA region, as shown in Table 2. In the city of Belo Horizonte (MG), the A2143G mutation was the most prevalent in a study in which 90.0% of the isolated resistant strains contained the point mutations A2143G and/or A2142G in the 23S rRNA region⁽⁵²⁾. In São Paulo (SP), studies found that the A2143G mutation was present in 67.0% and 58.3% of resistant strains isolated from children and adults respectively who had been prescribed clarithromycin. Both studies, although analyzing strains from patients of different age ranges, analyzed the same number (12) of clinical isolates and also found a lower prevalence of A2142G mutation (33.0% and 25.0%)^(26,91). Similarly, in northeastern Brazil, the A2143G point mutation was present in most *H. pylori* isolates (71.4%) carrying clarithromycin resistance genotype⁽⁴⁹⁾.

TABLE 2. Antibiotic resistance rate of *Helicobacter pylori* in Brazil (2000-2012)

Area	Year	N° Patient <i>H. pylori</i> positive	Clarithromycin		Amoxicillin	Metronidazole	Levofloxacin	Reference
			Phenotypic	Genotypic				
SP	2000	90	7%(6/90) (b)	NA	29%(26/90) (e)	42%(38/90) (e)	NA	Mendonça et al. ⁽⁵⁷⁾
MG	2002	202	9.8%(20/202) (c)	90.0% (18/20) A2142G/A2143G A2142G: 16.7% (3/18), A2143G: 66.6% (12/18), A2142G + A2143G: 16.7%(3/18)	NA	53%(107/202) (e)	NA	Magalhães et al. ⁽⁵²⁾
SP	2003	52	(c)	94.2% (49/52) A2142G/A2143G A2142G: 87.7%(43/49) A2143G:12.3%(6/49)	NA	NA	NA	Ribeiro et al. ⁽⁷⁵⁾
SP	2003	138	16%(23/138) (c)	NA	38%(54/138) (e)	55%(75/138) (e)	NA	Godoy et al. ⁽²⁸⁾
SP	2008-2009	45	27%(12/45) (b)	100%(12/12) A2142G/A2143G A2143G: 67% (8/12) A2142G:33%(4/12)	4%(2/45) (d)	13%(6/45) (d)	NA	Garcia e al. ⁽²⁶⁾
AM	2007-2008	41	NA	75.6% (31/41) 23S rRNA A2143G (3.3%), T2182C (12.9%), G2224A (6.45%), T2215C (61.3%), A2192G (3.3%), G2204C (6.4%), T2221C (6.4%)	NA	NA	NA	Barile et al. ⁽⁶⁾
SP	2011	39	8%(3/39) (b)	NA	0% (0/39) (a)	51%(20/39) (e)	23%(9/39) (b)	Eisig et al. ⁽²²⁾
SP	2003-2006	488	NA	2.5% (12/488) A2143G/A2142G A2142G: 25%(3/12) A2143G:58.3%(7/12) A2143G + A2142G: 8.7% (1/12)	NA	NA	NA	Suzuki et al. ⁽⁹⁰⁾
RS	2011-2012	54	11.1% (6/54) (b)	NA	1.9%(1/54) (b)	NA	NA	Picoli et al. ⁽⁷¹⁾

NA: not analyzed. (a) MIC ≥ 0.5 g/mL, (b) MIC $\geq 1\mu\text{g/mL}$, (c) MIC $\geq 2\mu\text{g/mL}$, (d) MIC $\geq 4\mu\text{g/mL}$, (e) MIC $\geq 8\mu\text{g/mL}$.

In contrast, in the Amazon, which is located in northern Brazil, Barile et al. (2010) studied the variability of the 23S rRNA gene sequences of 41 isolates from biopsies of patients with gastric pathologies, and identified the A2143G mutation in only 3.3% of cases, while three other mutations previously linked to cases of resistance were detected in 6.4% (G2224A), 12.9% (T2182C) and 61.3% (T2215C) of cases. Furthermore, three other types of mutations that are not characterized by any population were found in 16.1% of cases. In addition to the high prevalence of mutations of the 23S rRNA gene of *H. pylori* strains circulating in the Amazon (AM), genetic variability of these mutations was detected in individuals with gastric diseases (gastritis, peptic ulcer or gastric cancer),

demonstrating the need to regionally characterize the profile of these strains to provide correct therapy⁽⁶⁾.

Amoxicillin

Another drug that is used in combination with standard therapy is amoxicillin, a β -lactamic antibiotic. This class of drugs binds to the penicillin-binding protein (PBP), enzymes involved in the biosynthesis of the peptidoglycan layer of the bacterial cell wall. Unlike the majority of Gram-negative bacteria that have a major mechanism of resistance through the production of β -lactamases, β -lactamic resistance in *H. pylori* results from changes in PBP that determine one reduced affinity of PBP for β -lactams^(21,67,99).

Worldwide, the general resistance to amoxicillin is low⁽⁶⁹⁾. Studies conducted in both Europe and Africa have not identified amoxicillin resistance in clinical *H. pylori* isolates^(7,8,13,82,108). On the other hand, in Latin American countries, such as Chile, Paraguay and Venezuela, the rate of resistance has been found to vary between 0 and 2%.^(23,29,68)

In Brazil, studies that used phenotypic methods also found low rates of resistance to amoxicillin, at 0-4%^(22,26,71). However, as a result of the disproportionate use of these antibiotics, especially for the treatment of common respiratory tract infections, bacteria can develop a tolerance to the drug^(28,57).

Although research that has examined *H. pylori* isolates-related mutations in terms of resistance to amoxicillin are scarce compared to those conducted for clarithromycin, previous studies have shown that the presence of mutations of PBPs is especially linked with PBP1, PBP2, and PBP3⁽²¹⁾. Studies in Korea and Japan that analyzed the sequence of resistant strains revealed amino acid substitutions in PBP1, including Ser414 → Arg and Asn562 → Tyr^(41,76). They also showed that mutations in PBP1 and PBP3 provided greater resistance than PBP1 and PBP2 mutations, or mutation only in PBP1⁽⁷⁷⁾. These results demonstrated that amoxicillin resistance in *H. pylori* is closely associated with amino acid substitutions in the PBP1 region^(41,54,67,76).

Metronidazole

Metronidazole is used as an alternative to clarithromycin and amoxicillin when a patient has an allergy or resistance to these two drugs⁽¹⁸⁾. The mechanism of action of metronidazole involves an oxidation of the bacterial DNA causing disruption of the double helix and consequent cell death⁽⁸⁵⁾. *H. pylori* resistance to metronidazole occurs due to mutations in the gene encoding *rdxA*, a homologue of classical nitroreductase that is responsible for reducing nitroaromatic compounds, such as metronidazole, to a product that is toxic to the bacteria. Thus, resistance is a result of a loss of function by mutational inactivation⁽³⁰⁾.

In Europe, the primary resistance rates to metronidazole in *H. pylori* isolates range from 14.0% to 59.0% in Italy, Norway, and Poland^(7,46,70,80). In Asia and Africa, high rates of resistance have been observed at around 70%-85%^(8,73,82). Similarly, developing countries, such as Paraguay (32.6%), Uruguay (36.0%) and Chile (26.3%), have higher rates of resistance to metronidazole, and rates in southeastern Brazil are between 40%-55%^(22,23,28,52,57,66,68,100). These high rates of resistance may have been result of the frequency of use of metronidazole for the treatment of other infections, such as parasitic and gynecological infections, in these regions, which can result in the development of resistant strains of *H. pylori*⁽⁵⁵⁾.

As mentioned above, the inactivation of the *rdxA* gene is strongly associated with resistance to metronidazole; however, other resistance mechanisms may exist on this bacterium⁽³⁶⁾. There are resistant strains that have not show *rdxA* mutations in the gene, suggesting that other genes that encode a NAD(P)H flavin nitroreductase, such as *frxA*, or mechanisms, such as regulation of transcription of the *rdxA* gene and the overexpression of *hfa* efflux pump, could be involved in the generation of resistance^(30,36,44,96,101).

According to Jeong et al. (2001), metronidazole resistance development in Canada may be associated with inactivation *rdxA* or both *rdxA* and *frxA*⁽³⁷⁾. In Asia, *rdxA* inactivation was responsible for metronidazole resistance in 66% of the isolates of *H. pylori*, and the other 33% were resistant by inactivating both *rdxA* and *frxA*. Similar results were found in Malaysia, where rates were 89.1% for insertions/deletions of *rdxA* and/or *frxA*. However, in the same study, 10.8% of strains resistant to metronidazole exhibited no change in both *rdxA* and *frxA*, indicating that mutations in other redox enzymes could be involved in the resistance of the strains; as such, it can be considered that the inactivation of the *rdxA* gene is often, but not always, associated with the development of metronidazole resistance in *H. pylori* isolates^(62,96,98). Therefore, more studies are required to identify the prevalence of metronidazole resistance of this bacterium, and correlating with leading molecular basis of resistance in order to rationally delineate the therapeutic use of this antimicrobial in therapies involving anti-*H. pylori*.

Levofloxacin

Fluoroquinolones, such as levofloxacin, are used as an alternative to standard antibiotics to treat *H. pylori*. Many studies suggest that second-line “rescue” therapy for the eradication of *H. pylori* consists of levofloxacin, amoxicillin and an inhibitor of the proton pump. However, the use of levofloxacin should be limited only to “rescue” therapy in order to avoid a rapid increase in *H. pylori* resistance to the quinolone^(17,53). The fluoroquinolone mechanism of action operates by inhibiting DNA gyrase and topoisomerase, which control and modify the topological state of DNA in the cell by interfering with bacterial DNA replication. The mechanism of *H. pylori* resistance to the fluoroquinolone appears to be mainly associated with mutations of the *gyrA* gene, the gene encoding the subunit A of DNA gyrase^(27,61).

Resistance ratios of *H. pylori* to this drug have remained constant in Europe, at about 22.0%-29.0%^(80,110). However, the rates in Asia are more variable and range from 4.9%-19.5%^(8,89). Furthermore, the primary resistance in Taiwan has increased over time from 4.9% between 2000 and 2007, to 8.3% between 2008 and 2010, and 13.4% between 2011 and 2012. This has led to calls for new strategies to restrict the consumption of fluoroquinolones⁽⁵⁰⁾. In Latin American populations, the summary prevalence was 15%, and an existing study that was conducted in Brazil by Eisig et al. (2011) identified a 23.0% resistance to levofloxacin^(14,22).

Regarding the studies linking *H. pylori* resistance to levofloxacin and the presence of mutations in *gyrA*, can be identified as the main, the changes in Asp91, both in France and in Japan. However, in China, only 37.9% of the isolates resistant to levofloxacin were associated with Asp91 mutations, while 55.2% presented Asn87^(24,97,106). Thus, it is necessary to estimate the prevalence of levofloxacin and to investigate the molecular basis of levofloxacin resistance through genotypical methods, and to establish a rational use of levofloxacin for the treatment of infections caused by *H. pylori*.

FACTORS ASSOCIATED WITH RESISTANCE

Besides the mechanisms of resistance developed by *H. pylori* to the main antimicrobials used in the treatment of infection of the bacteria, other factors have been associated with resistance, such as the presence or absence of certain pathogenicity genes of bacteria, in addition to the gender and age group of the patients.

Among the pathogenicity genes of *H. pylori* that have been associated with resistance is the *cagA* gene, which encodes a highly immunogenic protein that can result in varying degrees of inflammatory response^(9,10). It has been established that the *vacA* gene has a mosaic combination of its alleles, which determines the production of the vacuolating cytotoxin (*VacA*) associated with the pathogenicity of the bacterium. In general, the strains of *s1/m1/i1* produce large amounts of vacuolizing cytotoxin, which is why this genotype seems to be associated with more severe pathologies. In contrast, the strains *s1/m2* and *s2/m2/i2* show moderate, little, or no cytotoxic activity respectively^(4,78,102). This knowledge is important since the presence of virulent genotypes can cause a change in the physiological environment of the stomach, leading to damage to the gastric epithelium and mucus layer, where *H. pylori* resides⁽¹⁰³⁾. Therefore, an increase in blood flow can assist in the diffusion of antibiotics that can reach higher concentrations in inflamed mucosa⁽⁹²⁾.

Studies have shown that strains carrying the *cagA* gene are at a higher density and have an intense inflammatory response in the gastric epithelial cells and, thus, can proliferate more rapidly than *cagA* negative strains^(5,31,105). As antibiotics are most active on the bacteria that grows quickly, *cagA*-positive strains are more susceptible to antimicrobial activity than negative *cagA* strains. Furthermore, *cagA* negative strains can become resistant by mutation under selective pressure of antimicrobials. Therefore, the absence of *cagA* can be a risk factor in the development of antimicrobial resistance^(11,92,94).

In order to study the risk ratio for failure and for therapeutic success between *cagA*-negative and *cagA*-positive strains, Suzuki et al. (2006), via meta-analysis, found that the presence of the *cagA* positive strain increased the likelihood that the treatment of *H. pylori cagA* would be successful by 11% in comparison to negative strains. Indeed, in the Netherlands, cure rates for *H. pylori* were found to be higher for patients with *H. pylori cagA* positive strains than for those with *cagA*-negative strains⁽¹⁰³⁾.

In addition to the differences in elimination rates between *cagA* positive and negative strains, research has found variations between the association of resistance and the presence of *vacA* alleles according to their degree of pathogenicity. According to the assessment of the association between *cagA* strains and the alleles of *vacA*, one study in the Netherlands showed that *cagA+/vacAs1* strains were more pathogenic and can proliferate faster than *cagA-/vacAs2* strains and, therefore, are more susceptible to antibiotic activity⁽¹⁰³⁾. In Spain, *H. pylori* strains resistant to clarithromycin were also more often associated with the *vacAs2/m2* genotype and were more likely to be *cagA* negative, indicating that resistance

to clarithromycin may be related to less pathogenic clinical isolates⁽²⁾. However, in Brazil, a significant association between resistance to clarithromycin and *vacAs1/m1* strains was detected⁽²⁶⁾. This was also detected by Rashed et al. (2014) in Pakistan⁽⁷³⁾. On the other hand, a study conducted in Italy did not identify any correlation between resistance to clarithromycin and bacterial genomic pattern and/or positivity *cagA*⁽²⁰⁾. As suggested by van Doorn et al. (2000), these data demonstrate the need for studies that evaluate the different *H. pylori* genotypes associated with regionalized antimicrobial resistance profiles, whereas, patterns of pathogenicity factors and association with antibiotic therapy has shown variation among countries⁽¹⁰³⁾.

In terms of the association between the presence of resistance to drugs and the gender of patients infected with *H. pylori*, studies have shown that women are more likely to have high rates of drug resistance to the treatments administered as part of *H. pylori* therapy. A significant difference between the genders in terms of clarithromycin resistance was observed in Japan, with a resistance rate of 19.2% among men compared to 27.0% among women⁽⁴³⁾. Accordingly, in Italy, females were found to be at a high risk of developing resistance to clarithromycin and metronidazole. This positive correlation between the resistance to metronidazole and the female gender can be explained by the fact that metronidazole is frequently administered for the treatment of gynecological infections^(51,52,109). However, with regard to resistance to levofloxacin, the association was significant with members of the male sex⁽⁸⁰⁾.

Studies that have analyzed the relationship between the ages of the patients infected with *H. pylori* have found similar results in terms of resistance to clarithromycin and metronidazole. For both antimicrobials, resistance is more common in children or young adults than it is in older members of the population^(2,52). These higher resistance rates in children may be due to the increased prescription of these drugs for respiratory and parasitic infections, which frequently occur in this age group⁽⁹³⁾. Thus, knowledge of the relationship between these factors and *H. pylori* resistance against antimicrobial agents may confer additional information for the ideal choice of eradication therapy for patients infected with *H. pylori*. However, Zhang et al. (2015) conducted a multivariate analysis that indicated that factors such as gender and age were independent factors in influencing antibiotic resistance⁽¹¹¹⁾.

CONCLUSION

The results of various studies throughout the world that have examined *H. pylori* resistance rates compared to the main drugs used in antimicrobial therapy vary significantly, confirming the hypothesis that antibacterial resistance is a highly local phenomenon. Therefore, it is important to understand the distribution of the levels of specific genes associated with antimicrobial resistance in order to ascertain how the characteristics of a given population can influence which therapy is the most appropriate.

Treatment failure has considerable cost implications/benefits for the National Health System in terms of drugs,

diagnostic tests, and medical visits. Therefore, the implementation of incentive policies to reduce the consumption of antibiotics can contribute to a reduction in the levels of resistance to the main antimicrobials used in the anti-*H. pylori* therapy.

Authors' contributions

Vianna JS: conducted a literature review, drafting and review of the manuscript. Ramis IB, Ramos DF, Von Groll A and Silva PEA: guidance in the writing and structuring of the manuscript and reviewing the manuscript.

Vianna JS, Ramis IB, Ramos DF, Von Groll A, Silva PEA. A resistência de *Helicobacter pylori* aos antimicrobianos. Arq Gastroenterol. 2016;53(4):215-23.

RESUMO - Contexto - *Helicobacter pylori* tem uma distribuição a nível mundial, e está associado a patogênese de várias doenças do sistema digestivo.

O tratamento para a erradicação deste microrganismo envolve a utilização de uma combinação de agentes antimicrobianos, tais como amoxicilina, metronidazol, claritromicina e levofloxacino, combinados com inibidores da bomba de prótons. Embora a terapia atual seja eficaz, uma elevada taxa de fracasso de tratamento tem sido observada, principalmente devido à aquisição de mutações pontuais, um dos principais mecanismos de resistência desenvolvida por *H. pylori*, relacionado com o uso frequente e/ou inadequado dos antibióticos. **Conclusão** - Esta revisão abordou uma visão geral da resistência às principais drogas utilizadas no tratamento de *H. pylori*, confirmando a hipótese de que a resistência bacteriana é um fenômeno altamente local e as características genéticas de uma dada população podem influenciar qual terapia é a mais apropriada.

DESCRITORES - *Helicobacter pylori*. Resistência microbiana a medicamentos. Mutação pontual.

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