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ORIGINAL ARTICLE

Observational Study

Detection of *Helicobacter pylori* resistance to clarithromycin and fluoroquinolones in Brazil: A national survey

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Abstract

AIM

To evaluate bacterial resistance to clarithromycin and fluoroquinolones in Brazil using molecular methods.

METHODS

The primary antibiotic resistance rates of *Helicobacter pylori* (*H. pylori*) were determined from November 2012 to March 2015 in the Southern, South-Eastern, Northern, North-Eastern, and Central-Western regions of Brazil. Four hundred ninety *H. pylori* patients [66% female, mean age 43 years (range: 18-79)] who had never been previously treated for this infection were enrolled. All patients underwent gastroscopy with antrum and corpus biopsies and molecular testing using GenoType HelicoDR (Hain Life Science, Germany). This test was performed to detect the presence of *H. pylori* and to identify point mutations in the genes responsible for clarithromycin and fluoroquinolone resistance. The molecular procedure was divided into three steps: DNA extraction from the biopsies, multiplex amplification, and reverse hybridization.

RESULTS

Clarithromycin resistance was found in 83 (16.9%) patients, and fluoroquinolone resistance was found in 66 (13.5%) patients. There was no statistical difference in resistance to either clarithromycin or fluoroquinolones (P = 0.55 and P = 0.06, respectively) among the different regions of Brazil. Dual resistance to clarithromycin and fluoroquinolones was found in 4.3% (21/490) of patients. The A2147G mutation was present in 90.4% (75/83), A2146G in 16.9% (14/83) and A2146C in 3.6% (3/83) of clarithromycin-resistant patients. In 10.8% (9/83) of clarithromycin-resistant samples, more than 01 mutation in the 23S rRNA gene was noticed. In fluoroquinolone-resistant samples, 37.9% (25/66) showed mutations not specified by the GenoType HelicoDR test. D91N mutation was observed in 34.8% (23/66), D91G in 18.1% (12/66), N87K in 16.6% (11/66) and D91Y in 13.6% (9/66) of cases. Among fluoroquinolone-resistant samples, 37.9% (25/66) showed mutations not specified by the GenoType HelicoDR test.

CONCLUSION

The *H. pylori* clarithromycin resistance rate in Brazil is at the borderline (15%-20%) for applying the standard triple therapy. The fluoroquinolone resistance rate (13.5%) is equally concerning.

Key words: *Helicobacter pylori*; Microbial drug resistance; Clarithromycin; Fluoroquinolones; Molecular diagnostic techniques

Core tip: Antibiotic resistance is the main cause of failure in the treatment of *Helicobacter pylori* (*H. pylori*) infection. Using molecular methods, this study investigated bacterial resistance to clarithromycin and fluoroquinolones in 490 adult patients recruited from five regions in Brazil. These patients had never been previously treated for *H. pylori* infection. Clarithromycin and fluoroquinolone resistance was found in 16.9% and 13.5% of patients, respectively. Resistance to both drugs was found in 4.3% of patients. The mean primary *H. pylori* clarithromycin resistance rate in Brazil is at the borderline for applying the standard triple therapy, and the primary fluoroquinolone resistance rate is concerning.

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INTRODUCTION

Helicobacter pylori (*H. pylori*) is the main etiologic agent of peptic ulcer and is recognized as the most important risk factor for adenocarcinoma and lymphoma of the mucosa-associated lymphoid tissue (MALT)[1]. Triple therapy, in which a combination of two antibiotics (amoxicillin and clarithromycin) and a proton pump inhibitor (PPI) is administered for 7 to 14 d, has been demonstrated to be an effective *H. pylori* infection treatment in different meta-analyses and has been recommended in national and international consensus meetings[2-5]. This regimen has, however, exhibited decreased effectiveness in recent years, with eradication rates lower than 80%, as reported in different studies[6,7].

Although factors including the lack of compliance, lifestyle habits such as smoking, Cag-negative strains, CYP2C19 genetic polymorphisms, altered immunity, and elevated bacterial load may all contribute to therapy failure, the main factor that causes therapy failure is bacterial resistance, especially to clarithromycin, metronidazole, and fluoroquinolones[8,9]. Similar to other bacterial species (*e.g.*, *Mycobacterium tuberculosis*), *H. pylori* acquires antibiotic resistance by chromosomal mutations, not by acquiring plasmids[10]. Although drug efflux proteins can contribute to the natural insensitivity to antibiotics and emerging antibiotic resistance, the main mechanism that contributes to *H. pylori* resistance

is vertically transmitted point mutations in the DNA[9-12].

Clarithromycin interacts with the peptidyl transferase in domain V of the 23S rRNA subunit, an interaction that suppresses bacterial ribosome activity and inhibits protein synthesis[9]. Point mutations at positions 2146 and 2147, formerly known as 2142 and 2143 (the numeration is from genome sequencing of GenBank NC000921 - J99 and NC000915 - HP 26695)[13], of the 23S rRNA gene have been shown to lead to a modification in ribosome conformation, which consequently reduces clarithromycin affinity and leads to bacterial resistance to the drug[9]. Three major point mutations in the 23S rRNA gene have been described to be responsible for over 90% of clarithromycin resistance cases observed in occidental countries[7]. These are A2146C (point mutation at position 2146 by substitution of adenine for cytosine), A2146G (point mutation at position 2146 by substitution of adenine for guanine), and A2147G (point mutation at position 2147 by substitution of adenine for guanine). Quinolone resistance on the other hand develops following point mutations in the DNA-gyrase enzyme involved in bacterial DNA replication[9]. *H. pylori* DNA gyrase comprises two subunits (gyrA and gyrB), and the mutations are found in a specific region of the *gyrA* gene called the quinolone resistance-determining region. Eleven mutations have been described, and these occur in codons 86, 87, 88, and 91[9]. The most frequently encountered mutations occur in codons 87 and 91[9,14,15], and these have been shown to be present in 80% to 100% of antibiotic resistance cases[16-18].

H. pylori antimicrobial resistance can be investigated in the laboratory by phenotypic and genotypic methods[7]. Bacterial culture and determination of the minimum inhibitory concentration (MIC) of the antibiotic are characteristics of the phenotypic method. In addition to providing a definite diagnosis of the infection, the phenotypic method also allows for drug sensitivity to be determined. Bacteria are, however, rarely cultured in medical practices due to the special care required in the transport of samples, the fastidious growth nature of *H. pylori*, and the need for appropriate culture media, which are most often unavailable[7].

Genotypic methods such as PCR, on the other hand, are increasingly used for bacterial detection and identification of point mutations[9]. European studies have recently validated a new molecular test that combines PCR and hybridization, allowing for the rapid detection of bacterial resistance to both clarithromycin and fluoroquinolones[13,19]. This novel GenoType HelicoDR (Hain Life Science, Germany) test involves a DNA strip coated with different specific primers (probes) designed to hybridize with the wild-type allele or reveal mutant sequences[13]. This method is faster than the phenotypic method and can be performed directly from endoscopic gastric biopsies, making the prior culture of the microorganism unnecessary[9].

Taking into consideration that primary resistance to *H. pylori* is a major factor for treatment failure in first-line *H. pylori* regimens, the Maastricht IV *H. pylori* Consensus Report recommends that the (PPI) clarithromycin-containing triple therapy, without prior susceptibility testing, should be abandoned when the clarithromycin resistance rate in the region is over 15%-20%[4]. Fluoroquinolones should be reserved for cases of retreatment or employed in high primary clarithromycin resistance areas[4]. However, the success of quinolones also decreases when there is bacterial resistance. It is important for clinicians to know the local prevalences of *H. pylori* resistance to clarithromycin and fluoroquinolones so that they can select the most appropriate *H. pylori* regime in first- and second-line eradication treatments. Therefore, the aim of this study was to assess the prevalence of primary *H. pylori* resistance to clarithromycin and fluoroquinolones in a large Brazilian population using the molecular GenoType HelicoDR test on gastric biopsy specimens.

MATERIALS AND METHODS

Patients

A multicenter study including the Southern (Porto Alegre, RS, Brazil), South-Eastern (Belo Horizonte, MG, Brazil), Northern (Manaus, AM, Brazil), North-Eastern (Salvador, BA and Maceio, AL, Brazil), and Central-Western (Goiania, GO, Brazil) regions of Brazil was designed. The recruitment of participants was proportional to the populations of the regions. A common protocol was adopted after approval by the Human Research Ethical Committee at all participant centers, and written consent was obtained prior to entering the study. From November 2012 through March 2015, enrolled patients never previously treated for *H. pylori* infection were submitted to diagnostic endoscopy and tested for *H. pylori* infection due to abdominal symptoms. All participants tested positive for *H. pylori* by a previously validated rapid serological test (Abon Biopharm, Hangzhou, China) performed immediately before the endoscopy[20].

Endoscopy and gastric biopsies

Four gastric biopsies (two from the antrum and two from the corpus) were taken during the endoscopy and were immediately immersed in micro-tubes containing RNAlater® (Ambion, Cat. # AM7020, United States), a solution that promotes immediate RNA stabilization and protection, thereby minimizing the need to immediately process the tissue samples. The samples were kept in a refrigerator at 4 ℃ before they were sent to the central laboratory of the study in Belo Horizonte where they were weighed and then frozen at -80 ℃ until the molecular tests were performed.

Molecular diagnostic technique

The molecular test GenoType HelicoDR was designed to identify the mutations A2146C, A2146G, and A2147G

Table 1 Distribution of patients by sampling region, gender, and age

Brazil - Region	Distribution by gender (% of females)	Distribution by age (average; minimum and maximum)
Northern (n = 36)	22 F; 14 M (61.1%)	41.4 (21-71)
North-Eastern (n = 138)	89 F; 49 M (64.5%)	37.7 (18-75)
Central-Western (n = 26)	13 F; 13 M (50.0%)	41.3 (21-65)
South-Eastern (n = 217)	145 F; 72 M (66.8%)	40.6 (19-76)
Southern (n = 73)	45 F; 28 M (61.6%)	58.0 (23-79)
Total (n = 490)	314 F; 176 M (64.1%)	42.4 (18-79)

F: Female; M: Male.

Table 2 Prevalence of *H. pylori* resistance to clarithromycin and fluoroquinolones in each region

	Northern (n = 36)	North-Eastern (n = 138)	Central-Western (n = 26)	South-Eastern (n = 217)	Southern (n = 73)	P value	Total (n = 490)
Clarithromycin resistance	16.6%	14.5%	19.2%	17.5%	19.1%	0.55	16.9% (95%CI: 13.7%-20.6%)
Fluoroquinolones resistance	2.7%	13.7%	15.4%	13.8%	16.4%	0.06	13.5% (95%CI: 10.6%-16.8%)

in the 23S rRNA gene and N87K, D91N, D91G, and D91Y in the gyrA gene. The test was divided into three steps: DNA extraction from the biopsy samples using the validated QIAmp DNA Mini Kit (Qiagen, Benelux, The Netherlands), multiplex amplification with biotinylated primers, and reverse hybridization using a specific incubator according to the manufacturer's instructions. The hybridization was performed on strips prepared at the Hain Lifescience factory that were coated with different specific oligonucleotides (DNA probes) using DNA strip technology.

Results regarding *H. pylori* detection and susceptibility to clarithromycin and fluoroquinolones were obtained by analyzing the positive and negative bands on the DNA strips. The most frequent mutations involved in resistance to the two antibiotics were also evaluated.

Statistical analysis

Descriptive statistics techniques, including central tendency and variability measures, were employed. The association between resistance/susceptibility to antimicrobials and the gender of the patients was evaluated using Fisher's Exact Test, and the association between resistance and age was evaluate, using Student's t-test. Resistance rates between regions were compared using Fisher's exact test. The odds ratios and 95% confidence intervals (CI) were used as risk estimates, and statistical significance was recognized at $P < 0.05$. The 22.0 version of IBM SPSS Statistics was used for the statistical calculations.

RESULTS

Five hundred nineteen patients were initially recruited for this study. Twenty-nine of them were subsequently excluded due to either the absence of *H. pylori* in the hybridization method (21 cases: 3 from the North-Eastern, 8 from the South-Eastern, 5 from the Northern, and 5 from the Central-Western regions) or the absence of a *gyrA* band (8 cases: 5 from the Central-Western, 1 from the Northern, 1 from the North-Eastern, and 1 from the Southern regions). The distribution of the remaining 490 patients according to gender, region, and age is shown in Table 1.

Clarithromycin and fluoroquinolone resistance were observed in 83 (16.9%; 95%CI: 13.7%-20.6%) and 66 (13.5%; 95%CI: 10.6%-16.8%) patients, respectively. Among the different centers, the rates of resistance ranged from 14.5% to 19.2% for clarithromycin and from 2.7% to 16.4% for fluoroquinolones. The differences were however not statistically significant for either the resistance ratios or *H. pylori* susceptibility (P = 0.55 and P = 0.06, respectively). Table 2 shows the results of general antibiotics resistance in each evaluated region.

More than one hybridization band (characterizing heterogeneous strains) was observed in the genes of 124 patients (25.3%): 11 patients for both the 23S and *gyrA* genes, 61 patients for the 23S gene, and 74 patients for the *gyrA* gene.

Resistance to clarithromycin was statistically significantly higher in women than in men (OR = 2.3, 95%CI: 1.3-4.0, P = 0.003). No statistically significant differences in fluoroquinolones resistance were found in the distribution between genders (P = 0.073).

In relation to age, no statistically significant differences were identified between patients with strains sensitive and resistant to clarithromycin (P = 0.796) and to fluoroquinolones (P = 0.176).

Among the 83 clarithromycin-resistant samples, 74.7% (62/83) exhibited heterogeneity in the 23S rRNA gene, 57.8% (48/83) in the *gyrA* gene, and 13.2% (11/83) in both genes. Among the 66 fluoroquinolone-resistant samples, 69.7% (46/66) exhibited heterogeneity in the *gyrA* gene, 22.7% (15/66) in the 23S rRNA gene, and 10.6% (7/66) in both genes.

Table 3 Distribution of mutations in the 23S gene in each region

Brazil - Region	Northern	North-Eastern	Central-Western	South-Eastern	Southern	Total
MUT1[1]	-	3	2	8	1	14
MUT2[2]	-	1	-	1	1	3
MUT3[3]	6	18	5	32	14	75
MUT2 + MUT3	-	1	-	1	-	2
MUT1 + MUT3	-	1	2	3	-	6
MUT1 + MUT2 + MUT3	-	-	-	-	1	1
Total mutations	6	24	9	45	17	101

[1]Mutation A2146G; [2]Mutation A2146C; [3]Mutation A2147G.

Table 4 Distribution of mutations in the gyrA gene in each region

Region	Northern	North-Eastern	Central-Western	South-Eastern	Southern	Total
Codon 87 mutant						
No ident[1]	-	6	1	10	2	19
Gyr87 MUT[2]	1	4	-	4	2	11
Total	1	10	1	14	4	30
Codon 91 mutant						
No ident[1]	-	2	-	4	2	8
MUT1[3]	-	7	2	9	5	23
MUT2[4]	-	2	-	7	3	12
MUT3[5]	-	1	1	4	3	9
Total		12	3	24	13	52
Multiple mutations in codon 91						
MUT1 + MUT3	-	1	1	-	2	4
MUT1 + MUT2	-	-	-	3	1	4
MUT1 + MUT2 + MUT3		-	-	1		1
Total	-	1	1	4	3	9
Codons 87 + 91 mutants	-	2	-	3	2	7
Total mutations	1	22	4	38	17	82

[1]Mutant codon not specified; [2]Mutation at codon N87K, nucleotide AAA; [3]Mutation at codon D91N, nucleotide AAT; [4]Mutation at codon D91G, nucleotide GGT; [5]Mutation at codon D91Y, nucleotide TAT.

Heteroresistance (wild-type band and mutant band in the same studied codon) was identified in 73.5% (61/83) of the clarithromycin-resistant samples and in 51.5% (34/66) of the fluoroquinolone-resistant samples.

In 37.9% (25/66) of patients whose samples were resistant to fluoroquinolones, mutations that were not specified by the GenoType HelicoDR test were found. There was an absence of both wild-type and mutant bands in codon 87 for 19 patients, as well as in codon 91 for 8 patients. Among these, 2 patients also exhibited the absence of bands in codon 87. After analyzing the above data by sampling region, 3 samples were from the South, 8 were from the North-Eastern, 13 were from the Southeast, and 1 was from the Central-Western. Six patients exhibited both clarithromycin and fluoroquinolone resistance. However, only the *gyrA* gene was involved in the non-specified mutation.

The most common mutation in the 23S rRNA gene was A2147G, present in 90.4% (75/83) of clarithromycin-resistant patients. The second most common mutation in the gene was A2146G, present in 16.9% (14/83) of mutations, and the third most common mutation was A2146C, present in 3.6% (3/83) clarithromycin-resistant patients (Table 3). In 10.8% (9/83) of clarithromycin-resistant samples, more than one mutation in the 23S rRNA gene was found, which might indicate cases of co-infection or two different mutations in the same strain. Among fluoroquinolone-resistant samples, 37.9% (25/66) showed mutations not specified by the GenoType HelicoDR test. The D91N mutation was observed in 34.8% (23/66) of cases, D91G in 18.1% (12/66), N87K in 16.6% (11/66), and D91Y in 13.6% (9/66). In 24.2% (16/66) of the resistant cases, there was more than one mutation involved; 13.6% (9/66) of resistant cases had more than one mutation in codon 91, and 10.6% (7/66) of cases had mutations in both codons 87 and 91 (Table 4).

Resistance to both antimicrobials in the same sample was found in 4.3% (21/490) of the cases. On the other hand, 73.9% (362/490) of the samples did not show resistance to any of the drugs tested.

DISCUSSION

Despite the knowledge accumulated regarding *H. pylori* infection, the therapeutic arsenal remains limited to a few drugs, and the eradication rates of the classic

triple therapy have exhibited a downward trend in the Western world[6,7,21]. Following proper adherence to the treatment, *H. pylori* resistance to antimicrobials is the main factor associated with treatment failure[7]. For this reason, knowing the local profile of *H. pylori* resistance may help in the selection of antimicrobials to optimize treatment for eradication.

Because the phenotypic methods present logistical difficulties for use in routine daily practice, validated and accurate molecular methods have become valuable tools in the evaluation of resistance to antimicrobials[13,21,22]. In this study, we used the easy to perform GenoType HelicoDR test. The equipment for carrying out this test is usually already available in molecular biology laboratories[18]. Comparative studies carried out in different countries, including Brazil, reported agreement above 90% between the phenotypic and genotypic methods[13,19,22-24].

This is the first study conducted in Brazil in which *H. pylori* genotypic resistance to clarithromycin and fluoroquinolones was evaluated in different regions of the country. The rate of resistance to clarithromycin found in our study (16.9%) is high but is still acceptable for the empirical use of clarithromycin-based regimens. The resistance rate to fluoroquinolones (13.5%) is also a concern for its use in empirical second-line regimens for eradicating *H. pylori*. The finding that women had a higher prevalence of primary resistance to clarithromycin in our study corroborates the values reported in other studies[25,26]. There is speculation that cross-resistance caused by previous use of macrolides may be related to the higher *H. pylori* clarithromycin resistance rate among women, as women generally consume more antibiotics than men[27]. Considering the high rates of metronidazole resistance observed in Brazil[28,29] our findings suggest that the association of this agent with clarithromycin or fluoroquinolones could promote reduction in the *H. pylori* eradication rates.

Despite not being the objective of the study, considering the study design, sample size calculation, and convenience samples, the study found no statistically significant differences between the various collection centers regarding the resistance ratios and *H. pylori* susceptibility ($P > 0.05$) to clarithromycin and fluoroquinolones in the huge Brazilian territory. However, specially designed studies for this purpose are still required.

In Brazil, only unicentric studies have been conducted to evaluate the primary resistance to anti-*H. pylori* agents, with varying results. Primary resistance rates to clarithromycin have ranged from 8% to 16% in South-Eastern Brazil using phenotypic methods[28,29]. A study carried out in North-Eastern Brazil reported a primary resistance rate of 16.5% using phenotypic and genotypic methods[23]. The primary resistance rate to fluoroquinolones has ranged from 11% (genotypic method)[30] to 23% (phenotypic method) in South-Eastern Brazil[31]. The differences between the rates found in previous unicentric studies and in the present work may be attributed to differences in sample size, presence of heteroresistance[32], and overall differences in the history of previous antimicrobial consumption in the studied regions[33].

In clarithromycin-resistant samples, the A2147G mutation (90.4%) was prevalent, as was found in previous Brazilian studies[23,28] and in studies in other countries, such as France (83.5%)[13] and Belgium (80%)[19]. Among the 13.5% (66/490) fluoroquinolone-resistant samples studied, 37.9% (25/66) presented a mutant codon that could not be identified by the GenoType HelicoDR method, and the D91N mutation was found in 34.8% (23/66) of samples.

Although there are no molecular studies with information on the distribution profile of *gyrA* gene mutations in Brazil, a recent Colombian study identified the following as major mutations: N87I (47.2%), D91N (30.1%), and N87K (13.2%)[34]. It can be speculated that the 37.9% of fluoroquinolone-resistant samples that were not specified by the method used in this study may represent the N87I mutation, which was predominant in the Colombian study and is not detected in the GenoTtype HelicoDR test. The proportions of the D91N and N87K mutations found in this study are similar to those observed in Colombia. Such findings reinforce the need for studies to investigate the regional variations in the mutation pattern of the *gyrA* gene. In samples with *gyrA* heterogeneity (mixture of wild-type and/or mutant bands), a high resistance rate (43.2%) was found, and it is likely that this polymorphism may represent different stages in the development of mutations[35].

Our study also identified the occurrence of multiple mutations for fluoroquinolones and clarithromycin. We observed multiple mutations for fluoroquinolones in 21.9% of samples (codons 87 and 91 or more than one mutation in codon 91), which are essentially the result of a variety of point mutations in several loci of the *gyrA* gene. The presence of multiple mutations in the 23S rRNA gene for clarithromycin was found in 8.9% of the samples. Similar results were also reported in Belgium, where multiple mutations were found in 25% of samples for fluoroquinolones and 12% for clarithromycin[19]. Finally, simultaneous resistance to both antibiotics was unusual (4.3%) in our study, similar to what was reported in South Africa (2.5%)[22], Hong Kong (3.7%)[36], Italy (1.6%)[37], and Spain (4%)[38].

In conclusion, the mean primary *H. pylori* clarithromycin resistance rate in Brazil is situated at the borderline (15%-20%) for applying the standard triple therapy. The primary fluoroquinolone resistance rate is also of growing concern. The genotypic method used is available, fast, and offers acceptable transportation conditions, and it can be used for continuous surveillance for the proper selection of drugs for anti-*H. pylori* therapy in different regions.

COMMENTS

Background

The therapeutic regimen anti- *Helicobacter pylori* (*H. pylori*) has exhibited a decreased effectiveness in recent years. *H. pylori* resistance to antimicrobial is the main factor associated with treatment failure. It is important for clinicians to know the local prevalences of *H. pylori* resistance to clarithromycin and fluoroquinolones to select the most appropriate *H. pylori* regime in first- and second-line eradication treatments.

Research frontiers

The main mechanism that contributes to *H. pylori* resistance is vertically transmitted point mutations in the DNA. This study assessed the prevalence of primary *H. pylori* resistance to clarithromycin and fluoroquinolones in a large Brazilian population by using the molecular test.

Innovations and breakthroughs

The genotypic method is faster than the phenotypic method and can be performed directly from endoscopic gastric biopsies, making the prior culture of the microorganism unnecessary. The set-up for carrying out this test is usually already available in molecular biology laboratories.

Applications

The rate of resistance to clarithromycin discovered in our study (16.9%) indicates a high, but is still acceptable for the empirical use of clarithromycin-based regimens. Resistance to fluoroquinolone (13.5%) also reveals a concern for its use in empirical second-line regimens in eradicating *H. pylori*. The genotypic method used is achievable, fast, offers acceptable transportation conditions, and can be used as continuous surveillance for the proper selection of drugs for anti-*H. pylori* therapy in different regions.

Peer-review

H. pylori treatment failure is due to resistance of many isolates to antibiotics used for eradication. Due to this it is important to know the local profile of *H. pylori* resistance, which may help to select optimal treatment. The methodology is sound and clear and the manuscript is well written. On the basis of the results this genotyping method used in this study could be recommended for the proper selection for anti-*H. pylori* therapy. This study is valuable from the practical point of view. It increases the knowledge about local *H. pylori* resistance to clarithromycin and fluoroquinolones.

REFERENCES

1 **Chey WD**, Wong BC. American College of Gastroenterology guideline on the management of Helicobacter pylori infection. *Am J Gastroenterol* 2007; **102**: 1808-1825 [PMID: 17608775 DOI: 10.1111/j.1572-0241.2007.01393.x]

2 **Malfertheiner P**, Megraud F, O'Morain C, Bazzoli F, El-Omar E, Graham D, Hunt R, Rokkas T, Vakil N, Kuipers EJ. Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report. *Gut* 2007; **56**: 772-781 [PMID: 17170018 DOI: 10.1136/gut.2006.101634]

3 **Coelho LG**, Maguinilk I, Zaterka S, Parente JM, do Carmo Friche Passos M, Moraes-Filho JP. 3rd Brazilian Consensus on Helicobacter pylori. *Arq Gastroenterol* 2013; **50**: [PMID: 23748591 DOI: 10.1590/S0004-28032013005000001]

4 **Malfertheiner P**, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, Gensini GF, Gisbert JP, Graham DY, Rokkas T, El-Omar EM, Kuipers EJ. Management of Helicobacter pylori infection--the Maastricht IV/ Florence Consensus Report. *Gut* 2012; **61**: 646-664 [PMID: 22491499 DOI: 10.1136/gutjnl-2012-302084]

5 **Fock KM**, Katelaris P, Sugano K, Ang TL, Hunt R, Talley NJ, Lam SK, Xiao SD, Tan HJ, Wu CY, Jung HC, Hoang BH, Kachintorn U, Goh KL, Chiba T, Rani AA. Second Asia-Pacific Consensus Guidelines for Helicobacter pylori infection. *J Gastroenterol Hepatol* 2009; **24**: 1587-1600 [PMID: 19788600 DOI: 10.1111/j.1440-1746.2009.05982.x]

6 **Graham DY**, Fischbach L. Helicobacter pylori treatment in the era of increasing antibiotic resistance. *Gut* 2010; **59**: 1143-1153 [PMID: 20525969 DOI: 10.1136/gut.2009.192757]

7 **Mégraud F**. H pylori antibiotic resistance: prevalence, importance, and advances in testing. *Gut* 2004; **53**: 1374-1384 [PMID: 15306603 DOI: 10.1136/gut.2003.022111]

8 **Mégraud F**, Lamouliatte H. Review article: the treatment of refractory Helicobacter pylori infection. *Aliment Pharmacol Ther* 2003; **17**: 1333-1343 [PMID: 12786627 DOI: 10.1046/j.1365-2036.2003.01592.x]

9 **Mégraud F**, Bénéjat L, Ontsira Ngoyi EN, Lehours P. Molecular Approaches to Identify Helicobacter pylori Antimicrobial Resistance. *Gastroenterol Clin North Am* 2015; **44**: 577-596 [PMID: 26314669 DOI: 10.1016/j.gtc.2015.05.002]

10 **Mégraud F**, Lehours P. Helicobacter pylori detection and antimicrobial susceptibility testing. *Clin Microbiol Rev* 2007; **20**: 280-322 [PMID: 17428887 DOI: 10.1128/CMR.00033-06]

11 **Zhang Z**, Liu ZQ, Zheng PY, Tang FA, Yang PC. Influence of efflux pump inhibitors on the multidrug resistance of Helicobacter pylori. *World J Gastroenterol* 2010; **16**: 1279-1284 [PMID: 20222174 DOI: 10.3748/wjg.v16.i10.1279]

12 **Leclercq R**, Courvalin P. Bacterial resistance to macrolide, lincosamide, and streptogramin antibiotics by target modification. *Antimicrob Agents Chemother* 1991; **35**: 1267-1272 [PMID: 1929280 DOI: 10.1128/AAC.35.7.1267]

13 **Cambau E**, Allerheiligen V, Coulon C, Corbel C, Lascols C, Deforges L, Soussy CJ, Delchier JC, Megraud F. Evaluation of a new test, genotype HelicoDR, for molecular detection of antibiotic resistance in Helicobacter pylori. *J Clin Microbiol* 2009; **47**: 3600-3607 [PMID: 19759218 DOI: 10.1128/JCM.00744-09]

14 **Moore RA**, Beckthold B, Wong S, Kureishi A, Bryan LE. Nucleotide sequence of the gyrA gene and characterization of ciprofloxacin-resistant mutants of Helicobacter pylori. *Antimicrob Agents Chemother* 1995; **39**: 107-111 [PMID: 7695290 DOI: 10.1128/AAC.39.1.107]

15 **Tankovic J**, Lascols C, Sculo Q, Petit JC, Soussy CJ. Single and double mutations in gyrA but not in gyrB are associated with low- and high-level fluoroquinolone resistance in Helicobacter pylori. *Antimicrob Agents Chemother* 2003; **47**: 3942-3944 [PMID: 14638505 DOI: 10.1128/AAC.47.12.3942-3944.2003]

16 **Bogaerts P**, Berhin C, Nizet H, Glupczynski Y. Prevalence and mechanisms of resistance to fluoroquinolones in Helicobacter pylori strains from patients living in Belgium. *Helicobacter* 2006; **11**: 441-445 [PMID: 16961806 DOI: 10.1111/j.1523-5378.2006.00436.x]

17 **Kim JM**, Kim JS, Kim N, Jung HC, Song IS. Distribution of fluoroquinolone MICs in Helicobacter pylori strains from Korean patients. *J Antimicrob Chemother* 2005; **56**: 965-967 [PMID: 16159928 DOI: 10.1093/jac/dki334]

18 **Nishizawa T**, Suzuki H, Umezawa A, Muraoka H, Iwasaki E, Masaoka T, Kobayashi I, Hibi T. Rapid detection of point mutations conferring resistance to fluoroquinolone in gyrA of Helicobacter pylori by allele-specific PCR. *J Clin Microbiol* 2007; **45**: 303-305 [PMID: 17122023 DOI: 10.1128/JCM.01997-06]

19 **Miendje Deyi VY**, Burette A, Bentatou Z, Maaroufi Y, Bontems P, Lepage P, Reynders M. Practical use of GenoType® HelicoDR, a molecular test for Helicobacter pylori detection and susceptibility testing. *Diagn Microbiol Infect Dis* 2011; **70**: 557-560 [PMID: 21696906 DOI: 10.1016/j.diagmicrobio.2011.05.002]

20 **Martins GM**, Sanches BSF, Moretzsohn, LD, Coelho, LGV. Rapid in-office H. pylori serologic test: an useful tool in areas with high H. pylori prevalence? *Helicobacter* 2013; **18**: 120

21 **Lee JW**, Kim N, Nam RH, Park JH, Choi YJ, Kim JM, Kim JS, Jung HC. GenoType HelicoDR test in the determination of antimicrobial resistance of Helicobacter pylori in Korea. *Scand J Gastroenterol* 2014; **49**: 1058-1067 [PMID: 24957849 DOI: 10.3109/00365521.2014.894117]

22 **Tanih NF**, Ndip RN. Molecular Detection of Antibiotic Resistance in South African Isolates of Helicobacter pylori. *Gastroenterol Res Pract* 2013; **2013**: 259457 [PMID: 23710166 DOI:

10.1155/2013/259457]
23 **Lins AK**, Lima RA, Magalhães M. Clarithromycin-resistant Helicobacter pylori in Recife, Brazil, directly identified from gastric biopsies by polymerase chain reaction. *Arq Gastroenterol* 2010; **47**: 379-382 [PMID: 21225149 DOI: 10.1590/S0004-28032010000400011]
24 **Garcia GT**, Aranda KR, Gonçalves ME, Cardoso SR, Iriya K, Silva NP, Scaletsky IC. High prevalence of clarithromycin resistance and cagA, vacA, iceA2, and babA2 genotypes of Helicobacter pylori in Brazilian children. *J Clin Microbiol* 2010; **48**: 4266-4268 [PMID: 20826649 DOI: 10.1128/JCM.01034-10]
25 **De Francesco V**, Giorgio F, Ierardi E, Zotti M, Neri M, Milano A, Varasano V, Luzza F, Suraci E, Marmo R, Marone A, Manta R, Mirante VG, de Matthaeis M, Pedroni A, Manes G, Pallotta S, Usai P, Liggi M, Gatto G, Peri V, Sacco R, Bresci G, Monica F, Hassan C, Zullo A. Primary clarithromycin resistance in Helicobacter pylori: the Multicentric Italian Clarithromycin Resistance Observational (MICRO) study. *J Gastrointestin Liver Dis* 2011; **20**: 235-239 [PMID: 21961089]
26 **De Francesco V**, Margiotta M, Zullo A, Hassan C, Giorgio F, Burattini O, Stoppino G, Cea U, Pace A, Zotti M, Morini S, Panella C, Ierardi E. Prevalence of primary clarithromycin resistance in Helicobacter pylori strains over a 15 year period in Italy. *J Antimicrob Chemother* 2007; **59**: 783-785 [PMID: 17329269 DOI: 10.1093/jac/dkm005]
27 **Nicolini P**, Nascimento JW, Greco KV, de Menezes FG. [Factors related to prescriptions of antibiotics in a public pharmacy in the Western region of the city of São Paulo]. *Cien Saude Colet* 2008; **13** Suppl: 689-696 [PMID: 21936173 DOI: 10.1590/S1413-81232008000700018]
28 **Prazeres Magalhães P**, De Magalhães Queiroz DM, Campos Barbosa DV, Aguiar Rocha G, Nogueira Mendes E, Santos A, Valle Corrêa PR, Camargos Rocha AM, Martins Teixeira L, Affonso de Oliveira C. Helicobacter pylori primary resistance to metronidazole and clarithromycin in Brazil. *Antimicrob Agents Chemother* 2002; **46**: 2021-2023 [PMID: 12019131 DOI: 10.1128/AAC.46.6.2021-2023.2002]
29 **Godoy AP**, Ribeiro ML, Benvengo YH, Vitiello L, Miranda Mde C, Mendonça S, Pedrazzoli J. Analysis of antimicrobial susceptibility and virulence factors in Helicobacter pylori clinical isolates. *BMC Gastroenterol* 2003; **3**: 20 [PMID: 12911839 DOI: 10.1186/1471-230X-3-20]
30 **Martins GM**, Sanches BS, Moretzsohn LD, Lima KS, Cota BD, Coelho LG. Molecular detection of clarithromycin and fluoroquinolones resistance in Helicobacter pylori infection, directly applied to gastric biopsies, in an urban brazilian population. *Arq Gastroenterol* 2016; 53: 113-117 [PMID: 27305419 DOI: 10.1590/S0004-28032016000200012]
31 **Eisig JN**, Silva FM, Barbuti RC, Navarro-Rodriguez T, Moraes-Filho JP, Pedrazzoli Jr J. Helicobacter pylori antibiotic resistance in Brazil: clarithromycin is still a good option. *Arq Gastroenterol* 2011; **48**: 261-264 [PMID: 22147131 DOI: 10.1590/S0004-28032011000400008]
32 **De Francesco V**, Zullo A, Ierardi E, Giorgio F, Perna F, Hassan C, Morini S, Panella C, Vaira D. Phenotypic and genotypic Helicobacter pylori clarithromycin resistance and therapeutic outcome: benefits and limits. *J Antimicrob Chemother* 2010; **65**: 327-332 [PMID: 20008044 DOI: 10.1093/jac/dkp445]
33 **Megraud F**, Coenen S, Versporten A, Kist M, Lopez-Brea M, Hirschl AM, Andersen LP, Goossens H, Glupczynski Y. Helicobacter pylori resistance to antibiotics in Europe and its relationship to antibiotic consumption. *Gut* 2013; **62**: 34-42 [PMID: 22580412 DOI: 10.1136/gutjnl-2012-302254]
34 **Trespalacios Rangel AA**. Estudio de la resistencia de *Helicobacter pylori* a los zantimicrobianos e implicaciones en las terapias de erradicación. Bogota: Tesis [Doctorado en Ciencias Biologicas] - Facultad de Ciencias de la Pontificia Universidad Javeriana, 2011
35 **Kao CY**, Lee AY, Huang AH, Song PY, Yang YJ, Sheu SM, Chang WL, Sheu BS, Wu JJ. Heteroresistance of Helicobacter pylori from the same patient prior to antibiotic treatment. *Infect Genet Evol* 2014; **23**: 196-202 [PMID: 24576534 DOI: 10.1016/j.meegid.2014.02.009]
36 **Hung KH**, Sheu BS, Chang WL, Wu HM, Liu CC, Wu JJ. Prevalence of primary fluoroquinolone resistance among clinical isolates of Helicobacter pylori at a University Hospital in Southern Taiwan. *Helicobacter* 2009; **14**: 61-65 [PMID: 19191898 DOI: 10.1111/j.1523-5378.2009.00655.x]
37 **Zullo A**, Perna F, Hassan C, Ricci C, Saracino I, Morini S, Vaira D. Primary antibiotic resistance in Helicobacter pylori strains isolated in northern and central Italy. *Aliment Pharmacol Ther* 2007; **25**: 1429-1434 [PMID: 17539982 DOI: 10.1111/j.1365-2036.2007.03331.x]
38 **Navarro-Jarabo JM**, Fernández-Sánchez F, Fernández-Moreno N, Hervas-Molina AJ, Casado-Caballero F, Puente-Gutierrez JJ, Pallares-Manrique H, Rodríguez-Ramos C, Fernández-Gutierrez C, Pérez-Aisa A, Rivas-Ruiz F, Montiel Quezel-Guerraz N. Prevalence of Primary Resistance of Helicobacter pylori to Clarithromycin and Levofloxacin in Southern Spain. *Digestion* 2015; **92**: 78-82 [PMID: 26227669 DOI: 10.1159/000435949]

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