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Mutations in the Helicobacter pylori 23S rRNA gene associated with clarithromycin resistance in patients at an endoscopy unit in Medellín, Colombia

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Abstract: Introduction: Clarithromycin is the first-line antibiotic for the treatment of Helicobacter pylori infection. Bacterial resistance is mainly due to the presence of specific mutations in the 23S ribosomal RNA (rRNA) gene. Objective: To determine the frequency of A2143G and A2142G specific mutations in the 23S rRNA gene associated with clarithromycin resistance of H. pylori in samples from patients with dyspeptic manifestations in Medellín, northwestern Colombia. Materials and methods: DNA was extracted from gastric biopsy samples of patients with dyspeptic manifestations seen at an endoscopy unit in Medellín between 2016 and 2017. PCR was performed to amplify the bacterial s and m vacA regions, and a region in the 23S rRNA gene. The presence of the A2142G and A2143G mutations was determined using the restriction fragment length polymorphism (RFLP) technique with the BbsI and BsaI enzymes, respectively. Results: The prevalence of infection was 44.2% (175/396), according to the histopathology report. The positive samples were analyzed and the three regions of the bacterial genome were amplified in 143 of the 175 samples. The A2143G and A2142G mutations were identified in 27 samples (18.8%, 27/143). The most frequent mutation was A2143G (81.5%, 22/27). Conclusions: We found a high prevalence of H. pylori mutations associated with clarithromycin resistance in the study population. Further studies are required to determine the bacterial resistance in the Colombian population in order to define first line and rescue treatments.

Key words: Helicobacter pylori; drug resistance; bacterial; clarithromycin; stomach neoplasms

1 Introduction

Helicobacter pylori is a Gram-negative bacillus belonging to the Helicobacteraceae family and exists in two forms, spiral and coccoid; it has multiple unipolar flagella and encodes enzymes such as catalase, oxidase, and urease, which allow it to adapt to the conditions of the gastric epithelium. It is a microaerophilic bacterium with specific nutritional requirements, which makes it difficult to culture in vitro [1,2].

In most cases, H. pylori is acquired within the family environment via the oral-oral and fecal-oral routes during the first five years of life [3]. It is estimated that 50% of the world's population is infected with this microorganism. In developing countries, the prevalence of infection in the general population ranges from 60% to 90%, while in developed

countries it ranges from 25% to 50%, reflecting the importance of socioeconomic factors as determinants of its prevalence [4,5].

Helicobacter pylori selectively colonizes the gastric epithelium. In most people, the infection is asymptomatic; however, it can trigger chronic inflammation and lead to peptic ulcers in about 10% of individuals. Two types of cancer have been associated with H. pylori infection: Mucosa-Associated Lymphoid Tissue (MALT) lymphoma, which occurs in 0.1% of cases, and gastric adenocarcinoma, which is present in 1–3% of patients and is the fifth most common cancer worldwide [6–8].

Treatment to eradicate H. pylori infection prevents the development of gastroduodenal conditions. The globally recommended regimen includes a proton pump inhibitor and two types of antimicrobials. In first-line treatment, amoxicillin and clarithromycin are most commonly used, while in second-line treatment, amoxicillin and levofloxacin are employed. However, widespread resistance to these antibiotics has made it difficult to eradicate the infection [9].

The primary mechanism of H. pylori resistance to clarithromycin is a decrease in the macrolide's affinity for the 50S subunit of the bacterial ribosome due to point mutations in nucleotides adjacent to domain V of the 23S rRNA gene [10,11]. The most common mutations have been reported at positions 2142 and 2143, involving substitutions of adenine (A) for guanine (G) (A2142G and A2143G) or A for cytosine (C) (A2142C); the A2144G substitution has also been reported, although less frequently [12–15]. The development of resistance has been facilitated by nonadherence to treatment and the use of macrolides to treat other infections, primarily of the respiratory tract [16].

Colombia has only one study evaluating the overall prevalence of H. pylori infection. Bravo et al. reported a prevalence of 69.1% in 8,652 histopathological reports from endoscopies of patients with dyspeptic symptoms in 16 regional hospitals in the southwest, north, central, central-west, and eastern regions of the country, which varied among regions within a range of 41.7% to 99.1% [17].

Nationwide, the high prevalence of infection correlates with the equally high incidence of gastric adenocarcinoma, which between 2010 and 2014 was the leading cause of cancer death in men and the third leading cause in women, with mortality rates of 16.84 and 8.73 per 100,000 inhabitants, respectively [18,19].

H. pylori infection has a significant impact on the lives of affected individuals and on the healthcare system; therefore, its diagnosis and treatment are very important [20]. Treatment regimens should be based on local resistance studies due to the heterogeneity of the infection's presentation across different populations. However, in Colombia, treatments based on studies from other countries continue to be prescribed [21], as well as regimens that have lost their efficacy due to bacterial resistance, even though the most commonly used regimens are those mentioned in the Maastricht Consensus [9,22].

In a meta-analysis of H. pylori resistance in Latin America, Camargo et al. reported a clarithromycin resistance rate of 18% (range: 2.7% to 60%) in Colombia, based on studies in Bogotá, Pereira, Armenia, Tumaco, Túquerres, and Manizales, suggesting that Colombia is one of the countries with the highest prevalence of resistance in Latin America [23]. This implies that the bacterium's susceptibility to other antibiotics must be evaluated in order to establish first-line and rescue regimens, given that there are few antibiotics effective for treating the infection [21,24].

H. pylori resistance to clarithromycin has been demonstrated in studies of antibiotic treatment efficacy. Castaño et al., for example, compared two standard clarithromycin treatment regimens—one lasting 7 days and the other 10 days—administered to patients in Medellín; they found that eradication rates in the intent-to-treat analysis, as determined by an optimized breath test, were 67.8% and 74.3%, respectively, with no statistically significant differences; these percentages are below optimal for eradication [25]. Subsequently, the same authors compared standard treatment with clarithromycin to a 10-day regimen with levofloxacin and found a significant difference in efficacy between the two regimens in favor of

levofloxacin [26].

In Medellín, the prevalence of H. pylori infection in symptomatic patients ranges from 36.4% to 65%; however, the prevalence of clarithromycin resistance in this bacterium is unknown [17,27].

The objective of this study was to determine the frequency of the A2143G and A2142G point mutations in the 23S rRNA gene associated with H. pylori resistance to clarithromycin in gastric biopsies from patients with dyspeptic symptoms treated at an endoscopy unit in Medellín.

2 Materials and methods

2.1 Study population

A descriptive cross-sectional study was conducted among patients over 18 years of age with dyspeptic symptoms treated at an endoscopy unit at IPS SURA (Industriales de Medellín campus) between February 2016 and April 2017. This care unit is a second-level facility and primarily serves patients in socioeconomic strata 4 through 6 who have prepaid health insurance plans.

A sample size of 175 patients was calculated based on an estimated prevalence of resistance of 16% for the Colombian population, with a 95% confidence interval and a 5% margin of error.

Patients included in the study had not received treatment with proton pump inhibitors or antibiotics in the month prior to sample collection. Those diagnosed with cancer, terminal illness, or a history of gastrectomy were excluded.

Each patient was given an explanation of the study, and if they agreed to participate, they were asked to sign an informed consent form.

Five gastric biopsies were taken for histopathological examination following the Sydney protocol: the greater and lesser curvatures of the antrum and body, and the angular incisura [28]; an additional biopsy was taken from the antrum for molecular analysis, as this is the region of the stomach with the highest bacterial density in cases of H. pylori infection [29]. The antrum sample was placed in a sterile 1.5-ml plastic tube containing brain-heart infusion (BHI, BD, Sparks, MD, USA) and 20% glycerol. The samples were refrigerated at 4 °C and transported to the Gastrohepatology Laboratory at the University of Antioquia on the same day as the endoscopic procedure, where they were stored at -70 °C until processing.

2.2 Ethical considerations

The project was approved by the Bioethics Committee for Human Research at the University Research Center of the University of Antioquia.

2.3 DNA extraction and PCR amplification

All biopsies obtained underwent histopathological analysis and were stained using standard hematoxylin and eosin staining. Approximately 70% of the samples were analyzed by a pathologist specializing in the gastrointestinal tract, and the remaining 30% were evaluated by other pathologists.

From the samples that tested positive for H. pylori according to the histopathology report, total DNA was extracted using a commercial kit (QIAamp DNA Mini Kit™, Qiagen, Germany) and following the manufacturer's instructions. The extracted material was quantified (NanoDrop 2000™, Thermo Scientific, USA).

To determine the presence of the bacterium in the sample, the s and m regions of the vacA gene were amplified using the primers described by Acosta et al.: vacA s: F 5'-ATGGAAATACAACAAACACAC-3' and R 5'-CTGCTTGAATGCG CCAAAC-3'; vacA m: F'-CAATCTGTCCAATCAAGCGAG-3' and R 5'-GCGTCTAAATAATTCCAAGG-3' [30]. In samples that tested positive for the s and m regions of the vacA gene, a region of the 23S rRNA gene was amplified to determine the presence of mutations, using the primers previously described by Occhialini et al.: K1 5'-CCACAGCGAT GTGGTCTCAG-3' and K2 5'-CTCCATAAGAGCCAAAGCCC-3' [31].

Each of the three PCR reactions was carried out in a final volume of 25 µl containing 1X buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 mM of each primer, 2.5 U of Biolase™ (Bioline, United Kingdom), and 2 µl of extracted DNA.

In a thermal cycler (C1000 Touch™, Thermal Cycler), an initial denaturation cycle was programmed at 94 °C for two minutes, followed by 35 amplification cycles with variations depending on the region of interest: at 94°C for one minute, at 55 °C for one minute and at 72 °C for one minute for the s region of the vacA gene; at 94 °C for one minute, at 52 °C for one minute, and at 72 °C for one minute for the m region of the vacA gene; and at 94 °C for 30 seconds, at 60 °C for 30 seconds, and at 72 °C for 30 seconds for the 23S rRNA region, with a final cycle at 72 °C for five minutes.

The amplification products were subjected to electrophoresis on 2.5% agarose gels stained with ethidium bromide. For PCR standardization, genomic DNA from the H. pylori strain NCTC 11637 was used as a positive control (kindly donated by Dr. Alba Alicia Trespalacios, professor at the Pontificia Universidad Javeriana), and sterile distilled water was used as the negative control. It should be noted that strain NCTC 11637 does not have mutations at nucleotides 2142 and 2143 and, therefore, was not included in the RFLP assays.

In the Restriction Fragment Length Polymorphism (RFLP) technique, the restriction enzymes BbsI and BsaI (New England Biolabs, USA) were used, along with 3.5 µl of the 23S rRNA gene PCR product in a final volume of 20 µl, according to the manufacturer's instructions.

When the A2142G mutation is present, digestion of the amplicons from the peptidyltransferase region of the 23S rRNA gene with the BbsI enzyme yields two fragments of approximately 93 and 332 base pairs, whereas digestion with the BsaI enzyme (A2143G) produces three fragments of 20, 300, and 105 base pairs (GenBank U27270).

Amplicons of the core Open Reading Frame (ORF) of the hepatitis B virus (HBV), obtained from the pJET-TH24_1.5 plasmid (GenBank FJ589065), were included as a positive control for the RFLPs [32]. Amplification of this region yields a fragment of approximately 748 bp, which was digested with the BbsI enzyme to produce two fragments of 608 and 140 bp, and with the BsaI enzyme to produce two fragments of 508 and 240 bp. This sequence was selected via restriction mapping using the BioEdit software, version 7.1.9. Amplicons from the HBV core ORF (nucleotides 1098 to 1846) were included in all RFLP assays (Figure 1).

Eighteen (12.5%) positive samples were randomly selected from the histopathology study to evaluate the reproducibility of the PCR results for the three regions (vacA s and m and 23S rRNA) and of the RFLP method.

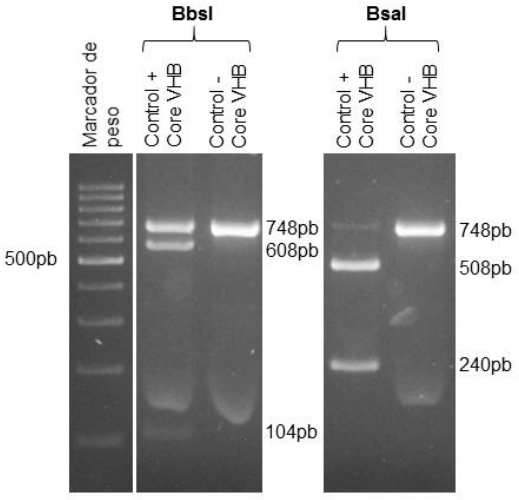

Figure 1. DNA electrophoresis on a 2.5% agarose gel stained with ethidium bromide. The bands obtained using the RFLP technique with the HBV Core ORF positive control are shown

In addition, 10 biopsies with negative histopathology results were analyzed by PCR to verify the presence of the vacA and 23S rRNA genes, and those with positive histopathology results but that did not amplify for vacA s and m and 23S rRNA were analyzed by PCR to verify genome integrity, using the following primers for the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene: 5'-CCTTCATTGACCTCAACTACATGG-3' and 5'-AGTCTTCTGGGTGGCAGTGATG G-3.

3 Results

Between February 2016 and April 2017, 588 patients referred for endoscopy due to dyspeptic symptoms presented at the IPS Sura in Medellín. Of these patients, 403 (68.5%) were invited to participate in the study, and 396 agreed; seven were excluded due to a history of gastric cancer or antibiotic use, or because they did not consent to the study. Fifty-nine percent (234/396) of the study participants were women; their ages ranged from 18 to 86 years, with a mean of 48 (±14) years.

Of the 396 cases included in the study, 175 tested positive for H. pylori infection based on histopathological evaluation with hematoxylin and eosin, representing an infection prevalence of 44.2% (175/396). It should be noted that other diagnostic methods, such as the rapid urease test or the urea breath test, as these are not routine tests for cases of dyspepsia at this healthcare facility.

Of the 175 samples that tested positive for H. pylori by histopathology, amplification of the s and m regions of the vacA gene and the 23S rRNA gene region was obtained in 143 (82%) (Figure 2). The vacA gene is a virulence factor that is present in all H. pylori strains, and its usefulness for detecting this bacterium in biological samples is comparable to that of other bacterial genes, such as ureA, ureC, 16S rRNA, 23S rRNA, and Hsp60 [30,33,34].

In 27 of 143 (18.8%) samples analyzed, the RFLP method identified the A2143G and A2142G point mutations in the 23S rRNA gene of H. pylori (Figures 2 and 3).

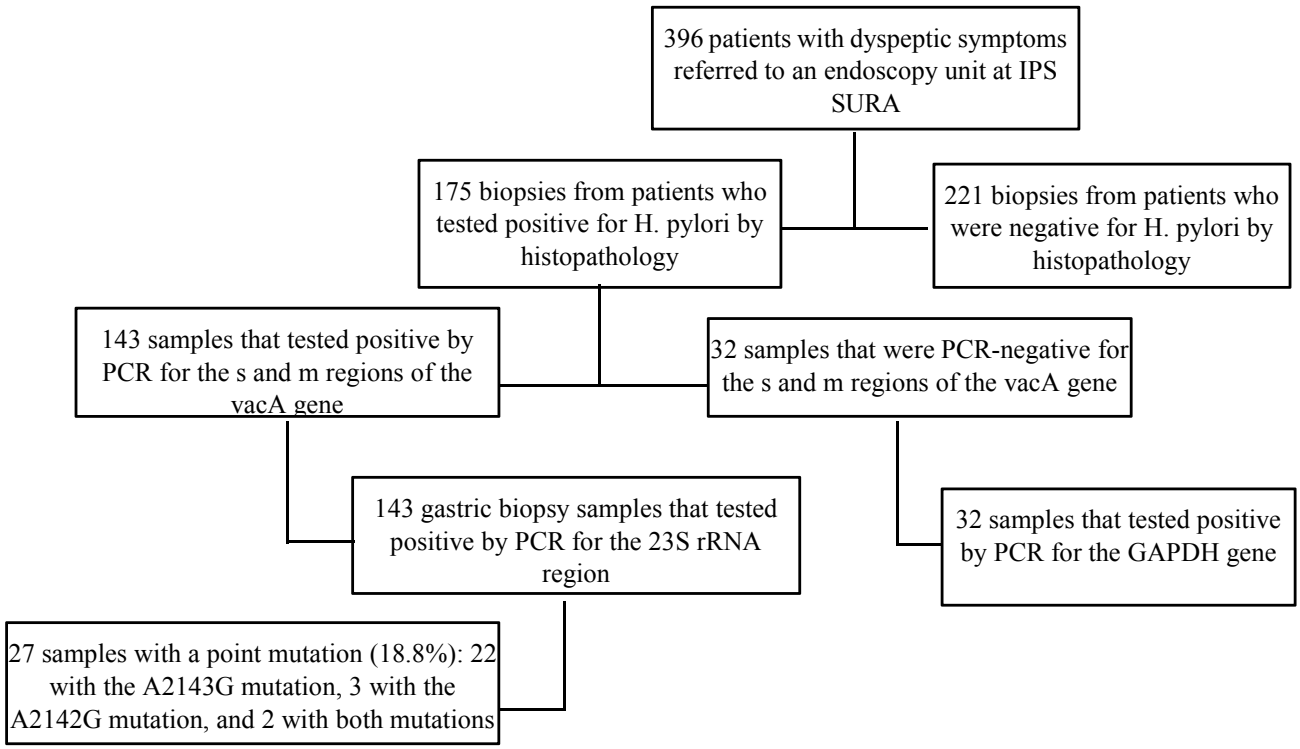

Figure 2. Diagram of the procedures and results obtained for the gastric biopsies processed in the study

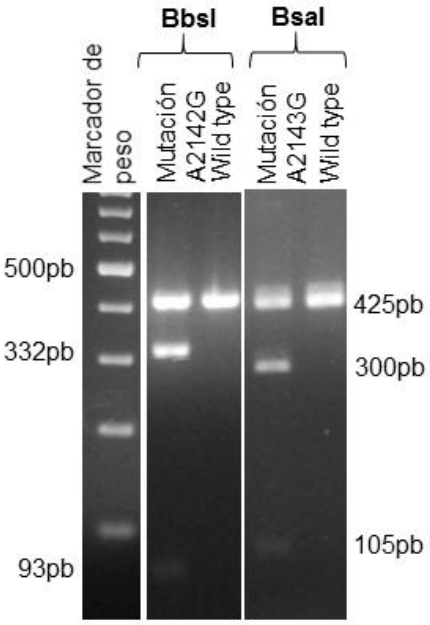

Figure 3. DNA electrophoresis on a 2.5% agarose gel stained with ethidium bromide. The bands obtained using the RFLP technique in the presence of the A2143G and A2142G mutations are shown

The most common substitutions were A2143G in 22 of 27 samples (81.5%) and A2142G in 3 of 27 (11%); both of these mutations were found simultaneously in 2 of 27 (7.4%) samples (Figure 2). Table 1 presents the demographic variables of patients with samples positive for H. pylori infection detected by PCR.

Follow-up information for the patients was not available; therefore, it was not possible to determine whether there was a correlation between the presence of mutations and resistance to treatment.

Eighty-one percent (116/143) of the analyzed samples in which amplification of bacterial genome sequences was achieved did not exhibit point mutations associated with clarithromycin resistance. The 18 samples analyzed in duplicate by PCR for the three regions of the bacterial genome—vacA s and m and 23S rRNA—and by the RFLP method demonstrated 100% reproducibility. Likewise, in none of the 10 samples that tested negative for H. pylori infection in the histopathological study were the regions of interest in the bacterial genome successfully amplified (data not shown).

Table 1. Demographic variables of patients with dyspeptic symptoms and samples positive for H. pylori infection by PCR treated at an endoscopy unit in Medellín

Characteristic	Mutations A2143G and A2142G	
	Presence n (%)	Absence n (%)
Sex		
Female	16 (59)	60 (52)
Male	11 (41)	56 (48)
Age (years)		
18–35	6 (22)	44 (38)
36–50	11 (41)	30 (26)
51–72	10 (37)	42 (36)
Total	27	116

4 Discussion

According to the histopathology report, the prevalence of H. pylori infection in the study population treated at the Medellín study unit between 2016 and 2017 was 44.2%. This prevalence is similar to that described in a retrospective study of samples obtained from patients with dyspeptic symptoms treated at the VID Diagnostic Clinic in Medellín between 2012 and 2013 (36.4%; 986/2708), a study in which the diagnosis of H. pylori infection was also determined by histopathology [27].

However, the results of both studies show a decrease in the prevalence of H. pylori infection compared to the 65% prevalence reported in histopathology reports from 494 patients at a public hospital in Medellín in 1997 [17]. This decrease is likely due to improvements in the population's sanitary conditions over the past two decades, as well as access to drinking water and wastewater collection and treatment [5].

Given the technical difficulties involved in isolating the bacterium, histopathological examination is considered one of the reference standard techniques for diagnosing H. pylori infection [1]. To minimize the likelihood of false negatives, standardized sampling must be performed, such as that described in the Sydney Protocol; furthermore, the use of antibiotics and proton pump inhibitors must be restricted for at least 30 days prior to endoscopy [35], in order to achieve better diagnostic sensitivity and specificity compared to other direct methods, such as the rapid urease test, the urea breath test, or PCR [36,37].

Given the technical difficulties involved in culturing the bacterium, PCR amplification from gastric biopsy specimens is a method that allows for the detection of H. pylori sequences; the sensitivity of PCR for H. pylori is estimated at 20 copies of the gene of interest per sample [38].

To determine H. pylori resistance to clarithromycin, various techniques have been implemented, such as agar diffusion, the epsilometry test, Fluorescent in situ Hybridization (FISH), RFLP, and real-time PCR [15]. A correlation has been reported between the detection of the A2142G and A2143G point mutations and resistance to clarithromycin, as determined by an increase in the Minimum Inhibitory Concentration (MIC) in culture using the agar dilution technique [12,13,39]. The RFLP method is faster and more cost-effective than other qualitative methods, such as sequencing and FISH, and has a specificity of 88%, a sensitivity of 85%, a positive predictive value of 54%, and a negative predictive value of 97% compared to culture [13]. Therefore, this technique represents an excellent alternative given the technical difficulty of isolating H. pylori [40–42]. Furthermore, the RFLP technique can be used on paraffin-embedded biopsies [43].

In the present study, the genotypic resistance of H. pylori to clarithromycin in the population was 18.8%. This percentage is similar to that reported in patients treated at healthcare facilities in other cities in Colombia. Trespalacios et al. reported a resistance rate of 13.6% in 256 samples from patients treated at a hospital in Bogotá between 2009 and 2011. Resistance was assessed using the agar dilution technique and confirmed by sequencing the bacterial 23S rRNA region, with at least one of the three mutations (A2143G, A2142G or A2142C) in samples with an increased MIC [14]. In previous studies, resistance rates of 15 to 17.7% were reported in samples from patients with dyspepsia treated at healthcare facilities in Bogotá between 2007 and 2009, based on results obtained using the epsilometry test and the disk diffusion method, respectively [16,44].

In a recent study at a hospital in Tumaco (southwestern Colombia), a resistance rate of 19.8% was determined using the agar dilution technique in 203 samples analyzed [45]. However, two other studies in Bogotá have reported higher rates of clarithromycin resistance. In the first of these studies, conducted in 2008 among 115 patients in a gastroenterology unit, a primary resistance rate of 60% was determined using the disk diffusion method, representing the highest rate of H. pylori resistance to clarithromycin reported to date in the country [46]. In a second study, clarithromycin resistance was detected

in 39.2% (42/107) of the samples using the agar dilution technique. Phenotypic resistance was confirmed by allele-specific PCR (Allele-Specific Polymerase Chain Reaction) in all resistant isolates, and the A2143G or A2142G mutations were identified [12].

On the other hand, resistance to this macrolide has been below 5% in patients from other cities in the country. In the Coffee Region (Pereira, Armenia, and Manizales), a prevalence of 3.8% was reported in 106 samples analyzed using the epsilometry test; of these, three had the A2143G mutation and one had the A2142G substitution as determined by PCR-RFLP analysis [47,48].

Acosta et al. evaluated the frequency of mutations associated with resistance by direct sequencing in 162 samples from patients treated at a hospital in Popayán (southwestern Colombia); 4.3% (7/162) of the samples had a mutation, and of these, four corresponded to the A2143G substitution and three to A2142G [30]. Bustamante et al. found the lowest rate of H. pylori resistance to clarithromycin reported in the country, 2.7%; this study was conducted on 149 isolates obtained from 206 patients treated at a hospital in Túquerres (southwestern Colombia) using the agar dilution technique [49]. In these studies, the most common point mutation was A2143G, which is consistent with the findings of the present study (81.5%).

In 116 of the 143 samples analyzed in another study, no point mutations were detected; however, this does not rule out bacterial resistance to this macrolide due to other mutations or other mechanisms, such as efflux pumps [39].

In 32 of the 175 histopathologically positive gastric biopsies in this study, none of the three regions of the bacterial genome (regions s and m of the vacA gene and a region of the 23S rRNA gene) could be amplified by PCR; it should be noted that PCR inhibitors were ruled out by amplifying the GAPDH gene (data not shown). The discrepancy between the histopathology and PCR results could be explained by the difference in the number of samples analyzed by histopathology (five biopsies from different regions of the stomach) and by PCR (a gastric antral biopsy), which increased the likelihood of detecting the bacilli via histopathology.

According to the meta-analysis by Camargo et al., in studies conducted in Argentina, Cuba, Ecuador, Brazil, Chile, Colombia, Costa Rica, Mexico, Peru, and Venezuela, the prevalence of H. pylori resistance to this macrolide ranged from 6% to 18%, with Colombia having the highest percentage (18%) [23]. In more recent studies, Squarcio et al. reported a resistance rate of 16.9% (83/490) using molecular methods in different regions of Brazil; the A2143G mutation was found in 90.4% of resistant isolates [50]. In Argentina, Zerbetto et al. found a resistance prevalence of 26.9%, and the most common mutation was A2143G, present in 89.4% of resistant isolates [51]. In Mexico, a resistance rate of 17.8% using the disk diffusion assay in 45 samples, and the A2143G mutation was detected in 1 of 8 isolates with phenotypic resistance [52].

When the rate of H. pylori resistance to clarithromycin in a population exceeds 15%, the second-line treatment is a quadruple regimen with bismuth or levofloxacin [9,23]. However, a recent study by Trespalacios et al. in Bogotá showed a considerable increase in levofloxacin-resistant isolates between 2009 (11.8%) and 2014 (27.3%) [53]. It is therefore of great importance to determine H. pylori to antibiotics, in order to establish treatment regimens based on the results in the Colombian population and thereby reduce the incidence of gastrointestinal diseases associated with the infection [54].

This is the first study to describe the status of H. pylori resistance to clarithromycin in patients treated in Medellín; a high prevalence of point mutations associated with this resistance was found. Given this finding, high rates of failure in eradicating the microorganism with triple therapy that includes this macrolide should be anticipated.

Limitations of this study included a lack of information on prior antibiotic use to treat H. pylori infection, making it impossible to distinguish between primary and secondary resistance, as well as a lack of details from the histopathology

report, such as bacterial load and the condition of the gastric epithelium; furthermore, study participants were treated at a single endoscopy unit in the city.

Further studies are needed to determine the bacteria's resistance to antibiotics in the Colombian population and to establish first-line and rescue treatments.

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Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

References

[1] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. IARC monographs on the evaluation of carcinogenic risks to humans. Volume 100. A review of human carcinogens. Part B. Biological agents. Lyon: International Agency for Research on Cancer; 2012. p. 385-435.

[2] Kao CY, Sheu BS, Wu JJ. Helicobacter pylori infection: An overview of bacterial virulence factors and pathogenesis. Biomed J. 2016;39:14-23. https://doi.org/10.1016/j.bj.2015.06.002.

[3] Zabala B, Lucero Y, Lagomarcino AJ, Orellana-Manzano A, George S, Torres JP, et al. Prevalence and dynamics of Helicobacter pylori infection during childhood. Helicobacter. 2017;22:e12399. https://doi.org/10.1111/hel.12399.

[4] Hunt RH, Xiao SD, Megraud F, León-Barua R, Bazzoli F, van Der Merwe S, et al. Helicobacter pylori in developing countries. World Gastroenterology Organisation Global Guideline. J Gastrointestin Liver Dis. 2011;20:299-304.

[5] Hooi JK, Lai WY, Ng WK, Suen MM, Underwood FE, Tanyingoh D, et al. Global prevalence of Helicobacter pylori Infection: Systematic review and meta-analysis. Gastroenterology. 2017;153:420-9. https://doi.org/10.1053/j.gastro.2017.04.022.

[6] Wroblewski LE, Peek RM, Wilson KT, Wilson KT. Helicobacter pylori and gastric cancer: Factors that modulate disease risk. Clin Microbiol Rev. 2010;23:713-39. https://doi.org/10.1128/CMR.00011-10.

[7] Gisbert JP. Enfermedades relacionadas con la infección por Helicobacter pylori. Gastroenterol Hepatol. 2013;36:39-50.

[8] Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. GLOBOCAN 2012 v1.0, Cancer incidence and mortality worldwide: IARC Cancerbase No. 11. Lyon: International Agency for Research on Cancer; 2013.

[9] Malfertheiner P, Megraud F, O'Morain CA, Gisbert JP, Kuipers EJ, Axon AT, et al. Management of Helicobacter pylori infection-the Maastricht V/Florence Consensus Report. Gut. 2017;66:6-30. https://doi.org/10.1136/gutjnl-2016-312288.

[10] Martínez JD, Henao SC, Lizarazo JI. Antibiotic resistance of Helicobacter pylori in Latin America and the Caribbean. Rev Colomb Gastroenterol. 2014;29:218-27.

[11] Versalovic J, Osato MS, Spakovsky K, Dore MP, Reddy R, Stone GG, et al. Point mutations in the 23S rRNA gene of Helicobacter pylori associated with different levels of clarithromycin resistance. J Antimicrob Chemother. 1997;40:283-6.

[12] Trespalacios AA, Rimbara E, Otero W, Reddy R, Graham DY. Improved allele-specific PCR assays for detection of clarithromycin and fluoroquinolone resistant of Helicobacter pylori in gastric biopsies: Identification of N87I mutation in GyrA. Diagn Microbiol Infect Dis. 2015;81:251-5. https://doi.org/10.1016/j.diagmicrobio.2014.12.003.

[13] Ciftci IH, Ugras M, Acartekin G, Asik G, Safak B, Dilek FH. Comparison of FISH, RFLP and agar dilution methods for testing clarithromycin resistance of Helicobacter pylori. Turkish J Gastroenterol. 2015;25:75-80. https://doi.org/10.5152/tjg.2014.4688.

[14] Trespalacios AA, Otero W, Caminos JE, Mercado MM, Ávila J, Rosero LE, et al. Phenotypic and genotypic analysis of clarithromycin-resistant Helicobacter pylori from Bogotá, D.C., Colombia. J Microbiol. 2013;51:448-52. https://doi.org/10.1007/s12275-013-2465-6.

[15] Kim KS, Kang JO, Eun CS, Han DS, Choi TY. Mutations in the 23S rRNA gene of Helicobacter pylori associated with clarithromycin resistance. J Korean Med Sci. 2002;17:599-603. https://doi.org/10.3346/jkms.2002.17.5.599.

[16] Trespalacios AA, Otero-Regino W, Mercado-Reyes M. Helicobacter pylori resistance to metronidazole, clarithromycin and amoxicillin in Colombian patients. Rev Colomb Gastroenterol. 2010;25:29-36.

[17] Bravo LE, Cortés A, Carrascal E, Jaramillo R, García LS, Bravo PE, et al. Helicobacter pylori: patología y prevalencia en biopsias gástricas en Colombia. Colomb Med. 2003;34:124-31.

[18] Lee YC, Liou JM, Wu MS, Wu CY, Lin JT. Eradication of Helicobacter pylori to prevent gastroduodenal diseases: Hitting more than one bird with the same stone. Therap Adv Gastroenterol. 2008;1:111-20. https://doi.org/10.1177/1756283X08094880.

[19] Instituto Nacional de Salud. Quinto Informe ONS: carga de enfermedad por enfermedades crónicas no transmisibles y discapacidad en Colombia. Quinta edición. Bogotá: Instituto Nacional de Salud; 2015.

[20] Regino WO. La importancia de cultivar Helicobacter pylori. Rev Colomb Gastroenterol. 2013;28:87-92.

[21] Sierra F, Forero JD, Rey M. Tratamiento ideal del Helicobacter pylori: una revisión sistemática. Rev Gastroenterol Mex. 2014;79:28-49. https://doi.org/10.1016/j.rgmx.2013.03.003.

[22]Gómez M, Ruíz O, Páramo-Hernández D, Albis R, Sabbagh LC. Erradicación del Helicobacter pylori: encuesta realizada por la Asociación Colombiana de Gastroenterología. Rev Col Gastroenterol. 2015;30:25-31.

[23] Camargo MC, García A, Riquelme A, Otero W, Camargo CA, Hernández-García T, et al. The problem of Helicobacter pylori resistance to antibiotics: A systematic review in Latin America. Am J Gastroenterol. 2014;109:485-95. https://doi.org/10.1038/ajg.2014.24.

[24] Di Ciaula A, Scaccianoce G, Venerito M, Zullo A, Bonfrate L, Rokkas T, et al. Eradication rates in italian subjects heterogeneously managed for Helicobacter pylori infection. Time to abandon empiric treatments in Southern Europe. J Gastrointest Liver Dis. 2017;26:129-37. https://doi.org/10.15403/jgld.2014.1121.262.itl.

[25] Castaño R, Ruiz-Vélez MH, Martínez-Hincapié C, Naranjo-Aristizábal FA, Campuzano-Maya G, Sanín-Fonnegra E, et al. Evaluación para comparar dos esquemas de terapia estándar (7 frente a 10 días) contra el Helicobacter pylori, con seguimiento clínico a 1 año. Rev Colomb Gastroenterol. 2012;27:80-7.

[26] Castaño R, Ruiz-Vélez MH, Campuzano-Maya G, Sanín-Fonnegra E, Puerta-Díaz JD, Calvo-Betancur V, et al. Randomized study comparing standard first line 10 day therapy against Helicobacter pylori including clarithromycin versus standard first line therapy with levofloxacin. Rev Colomb Gastroenterol. 2013;28:101-8.

[27] Correa S, Cardona AF, Correa T, Correa LA, García HI, Estrada S. Prevalence of Helicobacter pylori and histopathological features in gastric biopsies from patients with dyspeptic symptoms at a referral center in Medellín. Rev Colomb Gastroenterol. 2016;31:9-15.

[28] Peixoto A, Silva M, Pereira P, Macedo G. Biopsies in gastrointestinal endoscopy: When and how. GE Port J Gastroenterol. 2016;23:19-27. https://doi.org/10.1016/j.jpge.2015.07.004.

[29] Genta RM, Graham DY. Comparison of biopsy sites for the histopathologic diagnosis of Helicobacter pylori: A

topographic study of H. pylori density and distribution. Gastrointest Endosc. 1994;40:342-5. https://doi.org/10.1016/S0016-5107(94)70067-2.

[30] Acosta CP, Hurtado FA, Trespalacios AA. Determinación de mutaciones de un solo nucleótido en el gen 23S rRNA de Helicobacter pylori relacionadas con resistencia a claritromicina en una población del departamento del Cauca, Colombia. Biomédica. 2013;34:156-62. https://doi.org/10.7705/biomedica.v34i0.1649.

[31] Occhialini A, Urdaci M, Doucet-Populaire F, Bébéar CM, Lamouliatte H, Mégraud F. Macrolide resistance in Helicobacter pylori: Rapid detection of point mutations and assays of macrolide binding to ribosomes. Antimicrob Agents Chemother. 1997;41:2724-8. https://doi.org/10.1128/AAC.41.12.2724.

[32] Rendón JC, Cortés-Mancera F, Restrepo-Gutiérrez JC, Hoyos S, Navas MC. Molecular characterization of occult hepatitis B virus infection in patients with end-stage liver disease in Colombia. PLoS One. 2017;12:e0180447. https://doi.org/10.1371/journal.pone.0180447.

[33] Patel SK, Pratap CB, Jain AK, Gulati AK, Nath G. Diagnosis of Helicobacter pylori: What should be the gold standard? World J Gastroenterol. 2014;20:12847-59. https://doi.org/10.3748/wjg.v20.i36.12847.

[34] Jiménez F, Barbaglia Y, Bucci P, Tedeschi FA, Zalazar FE. Detección molecular y genotipificación de Helicobacter pylori en biopsias gástricas de pacientes adultos sintomáticos de la ciudad de Santa Fe, Argentina. Rev Argent Microbiol. 2013;45:39-43.

[35] Wang YK, Kuo FC, Liu CJ, Wu MC, Shih HY, Wang SS, et al. Diagnosis of Helicobacter pylori infection: Current options and developments. World J Gastroenterol. 2015;21:11221- 35. https://doi.org/10.3748/wjg.v21.i40.11221.

[36] de Martel C, Plummer M, van Doorn LJ, Vivas J, López G, Carillo E, et al. Comparison of polymerase chain reaction and histopathology for the detection of Helicobacter pylori in gastric biopsies. Int J Cancer. 2010;126:1992-6. https://doi.org/10.1002/ijc.24898.

[37] Cosgun Y, Yildirim A, Yucel M, Karakoc AE, Koca G, Gonultas A, et al. Evaluation of invasive and noninvasive methods for the diagnosis of Helicobacter pylori infection. Asian Pac J Cancer Prev. 2016;17:6165-72. https://doi.org/10.22034/APJCP.2016.17.12.5265.

[38] Hammar M, Tyszkiewicz T, Wadström T, O'Toole PW. Rapid detection of Helicobacter pylori in gastric biopsy material by polymerase chain reaction. J Clin Microbiol. 1992;30:54-8.

[39] Ahmad N, Zakaria WR, Abdullah SA, Mohamed R. Characterization of clarithromycin resistance in Malaysian isolates of Helicobacter pylori. World J Gastroenterol. 2009;15:3161-5. https://doi.org/10.3748/wjg.15.3161.

[40] Yula E, Nagiyev T, Kaya ÖA, Inci M, Çelik MM, Köksal F. Detection of primary clarithromycin resistance of Helicobacter pylori and association between cagA + status and clinical outcome. Folia Microbiol (Praha). 2013;58:141-6. https://doi.org/10.1007/s12223-012-0192-8.

[41] Mahmoudi S, Mamishi S, Banar M, Keshavarz Valian S, Bahador A, Najafi M, et al. Antibiotic susceptibility of Helicobacter pylori strains isolated from Iranian children: High frequency of A2143G point mutation associated with clarithromycin resistance. J Glob Antimicrob Resist. 2017;10:131-5. https://doi.org/10.1016/j.jgar.2017.04.011.

[42] Abdollahi H, Savari M, Zahedi MJ, Moghadam SD, Abasi MH. Detection of A2142C, A2142G, and A2143G mutations in 23s rRNA gene conferring resistance to clarithromycin among Helicobacter pylori isolates in Kerman, Iran. Iran J Med Sci. 2011;36:104-10.

[43] Pourakbari B, Mahmoudi S, Parhiz J, Sadeghi RH, Monajemzadeh M, Mamishi S. High frequency of metronidazole and clarithromycin-resistant Helicobacter pylori in formalin-fixed, paraffin-embedded gastric biopsies. Br J Biomed Sci. 2018;75:61-5. https://doi.org/10.1080/09674845.2017.1391466.

[44] Henao SC, Quiroga A, Martínez JD, Otero W. Resistencia primaria a la claritromicina en aislamientos de Helicobacter pylori. Rev Colomb Gastroenterol. 2009;24:110-4.

[45] Figueroa M, Cortés A, Pazos A, Bravo LE. Susceptibilidad in vitro de Helicobacter pylori a amoxicilina y claritromicina obtenido a partir de biopsias gástricas de pacientes de zona de bajo riesgo para cáncer gástrico. Biomédica. 2012;32:32-42. https://doi.org/10.7705/biomedica.v32i1.454.

[46] Yepes CA, Rodríguez A, Ruiz Á, Ariza B. Antibiotics resistance of Helicobacter pylori at the San Ignacio University Hospital in Bogotá. Acta Medica Colomb. 2008;33:11-4.

[47] Álvarez A, Moncayo JI, Santacruz JJ, Corredor LF, Reinosa E, Martínez JW, et al. Resistencia a metronidazol y claritromicina en aislamientos de Helicobacter pylori de pacientes dispépticos en Colombia. Rev Med Chil. 2009;137:1309-14. https://doi.org/10.4067/S0034-98872009001000005.

[48] Álvarez A, Moncayo JI, Santacruz JJ, Santacoloma M, Corredor LF, Reinosa E. Antimicrobial susceptibility and mutations involved in clarithromycin resistance in Helicobacter pylori isolates from patients in the western central region of Colombia. Antimicrob Agents Chemother. 2009;53:4022-4. https://doi.org/10.1128/AAC.00145-09.

[49] Bustamante-Rengifo JA, Matta AJ, Pazos A, Bravo LE. In vitro effect of amoxicillin and clarithromycin on the 3' region of cagA gene in Helicobacter pylori isolates. World J Gastroenterol. 2013;19:6044-54. https://doi.org/10.3748/wjg.v19.i36.6044.

[50] Sanches BS, Martins GM, Lima K, Cota B, Moretzsohn LD, Ribeiro LT, et al. Detection of Helicobacter pylori resistance to clarithromycin and fluoroquinolones in Brazil: A national survey observational study. World J Gastroenterol. 2016;22:7587-94. https://doi.org/10.3748/wjg.v22.i33.7587.

[51] Zerbetto G, Mendiondo N, Wonaga A, Viola L, Ibarra D, Campitelli E, et al. Occurrence of mutations in the antimicrobial target genes related to levofloxacin, clarithromycin, and amoxicillin resistance in Helicobacter pylori isolates from Buenos Aires City. Microb Drug Resist. 2016;23:351-8. https://doi.org/10.1089/mdr.2015.0361 .

[52] Alarcón-Millán J, Fernández-Tilapa G, Cortés-Malagón EM, Castañón-Sánchez CA, De Sampedro-Reyes J, Cruz-del Carmen I, et al. Clarithromycin resistance and prevalence of Helicobacter pylori virulent genotypes in patients from Southern México with chronic gastritis. Infect Genet Evol. 2016;44:190-8. https://doi.org/10.1016/j.meegid.2016.06.044.

[53] Trespalacios-Rangel AA, Otero W, Arévalo-Galvis A, Poutou-Piñales RA, Rimbara E, Graham DY. Surveillance of levofloxacin resistance in Helicobacter pylori isolates in Bogotá-Colombia (2009-2014). PLoS One. 2016;11:e0160007. https://doi.org/10.1371/journal.pone.0160007.

[54] Thung I, Aramin H, Vavinskaya V, Gupta S, Park JY, Crowe SE, et al. Review article: The global emergence of Helicobacter pylori antibiotic resistance. Aliment Pharmacol Ther. 2016;43:514-33. https://doi.org/10.1111/apt.13497.