Helicobacter pylori susceptibility to six commonly used antibiotics in Colombia

José Danilo Atehortúa-Rendón, BSc,[1]* ⓘ   Alonso Martínez, MSc, PhD,[2] ⓘ   Tania Liseth Pérez-Cala, MSc.[3] ⓘ

[1] Microbiologist and Bioanalyst, Grupo Bacteria & Cancer, Faculty of Medicine, Universidad de Antioquia; Medellín, Colombia.
[2] Biologist, Gruppo Bacteria & Cancer, Department of Microbiology and Parasitology, Faculty of Medicine, Universidad de Antioquia; Medellín, Colombia.
[3] Bacteriologist, Grupo Bacteria & Cancer, Faculty of Medicine, Universidad de Antioquia; Medellín, Colombia.

*Correspondence:
José Danilo Atehortúa-Rendón, MD
danilo.atehortua@udea.edu.co

Abstract

Helicobacter pylori (*H. pylori*) is a microaerophilic gram-negative bacillus that colonizes the gastric mucosa. It infects more than half the world's population, making it the most common bacterial infection. The prevalence of infection and associated diseases is high in developing countries. The recommended treatment for its eradication is triple therapy; however, its efficacy has decreased due to the lack of knowledge of the bacterial susceptibility pattern among the medical staff and the emergence of resistant strains. *H. pylori* susceptibility is associated with the bacteria's ability to adapt to hostile environments and the use of antibiotics. In Colombia, it has been reported that *H. pylori* is resistant to amoxicillin, metronidazole, clarithromycin, furazolidone, levofloxacin, and tetracycline. Studies on the susceptibility pattern have determined that the frequency of *H. pylori* susceptibility is variable and demonstrate the lack of data in most of the Colombian territory. With this in mind, the objective of this review is to describe the percentage of resistance to amoxicillin, metronidazole, clarithromycin, furazolidone, levofloxacin and tetracycline, which are used for the treatment of *H. pylori* infection, according to studies conducted in Colombia.

Keywords

Helicobacter pylori; Antimicrobial resistance; Gastroduodenal disorders; Gastric cancer.

INTRODUCTION

Helicobacter pylori (*H. pylori)* is a gram-negative, microaerophilic, and pleomorphic bacillus, approximately 3.5 μm in length and 0,5 μm in diameter, that affects about half of the world's population (1, 2). Infection reports vary, with a prevalence of 79.1% in Africa, 63.4% in Latin America and the Caribbean, 54.7% in Asia, 37.1% in North America, and 24.1% in Oceania (3, 4).

H. pylori infection prevalence is higher in developing countries, provided that there is a well-established association between its onset, socioeconomic level and hygiene conditions (3, 5). Its form of transmission is unclear, but it has been suggested that it is acquired in childhood by the fecal-oral route, and that intrafamily transmission is more frequent (6). Other studies suggest it is also transmitted by consuming contaminated water or vegetables (7, 8).

In 1994, the World Health Organization (WHO), through the *International Agency for Research on Cancer* (IARC), classified *H. pylori* as a type I carcinogen (9). H. pylori Infection is the main risk factor for the development of gastric cancer (GC), with a significant positive correlation and a relative risk of 3.8 (10). GC was the cause of death of 782,685 people worldwide in 2018 (10, 11).

Colombia has a high incidence of H. pylori infection (12). Its management is based on the international consensus of triple therapy (proton pump inhibitor, amoxicillin, and clarithromycin or metronidazole) (13). In addition, a guide for the treatment of H. pylori infections was published in 2015 in the country. However, this is not widely used by doctors and should be adjusted according to local resistance rates (14).

Therapeutic failure (TF) in H. pylori eradication is multifactorial and involves genetic, bacterial, and external factors of the patient, such as non-adherence to treatment schedule. Thus, the main cause of TF is bacterial resistance to antibiotics, which is acquired during treatment (15). Antibiotic resistance occurs because of the ability of microorganisms to adapt naturally to hostile media. It is also intrinsic to the absence of the drug binding site and acquired by genetic changes. In addition, *H. pylori* is capable of achieving genetic diversity by homologous mutations and recombinations (16-23). The emergence of resistant strains is also associated with the indiscriminate use of antibiotics and the lack of adherence to treatment as a result of the adverse effects derived from their use.

Currently, in Colombia, there are reports on *H. pylori* resistance to amoxicillin, metronidazole, clarithromycin, furazolidone, levofloxacin, moxifloxacin, and tetracycline (12, 24-29). In this context, the aim of this review is to describe the patterns of resistance to these antibiotics (amoxicillin, metronidazole, clarithromycin, furazolidone, levofloxacin and tetracycline) that have been described in the treatment of H. pylori infection by studies conducted in Colombia.

MATERIALS AND METHODS

Search Strategy and Selection Criteria

Searches were performed in the PubMed (National Library of Medicine of the United States, Bethesda, MD), LILACS (Latin American Literature of Information in Health Sciences: http://lilacs.bvsalud.org/en) and SciELO (electronic scientific library: http://www.scielo.org) databases. Articles were searched using the following strategy: *Helicobacter pylori, AND* Resistencia *AND* Colombia in Spanish, and *Helicobacter pylori, AND* drug resistance *AND* Colombia in English.

Inclusion Criteria

Original full-text available articles describing on H. pylori resistance to antibiotics in Colombia, regardless of their year of publication, were selected. From these studies, information such as the first author, the year of publica-

tion, the place where the research was conducted, the year samples were collected, the antibiotics evaluated, the number of samples (gastric biopsies or H. pylori isolates *)*, the prevalence of antibiotic resistance, *and* the resistance assessment method (epsilometry test [E-test], disk diffusion or polymerase chain reaction mutation detection [*Polymerase Chain Reaction*, PCR]) were obtained.

Exclusion criteria

Literature reviews, duplicated studies, and papers that did not have the necessary information were excluded. Reports in which H. pylori resistance to antibiotic treatment was not addressed were also excluded.

RESULTS

The search strategy in Spanish and English yielded 39 articles. Of these, 23 met the inclusion criteria. The following is a flowchart of the search and the selection process of the studies finally included in the review after the search strategy was applied (**Figure 1**). In addition, a summary table with the studies on H. pylori resistance conducted in Colombia is also shown (**Table 1**).

Amoxicillin

Amoxicillin is a first-choice antibiotic to treat *H. pylori* infection due to the high sensitivity of the microorganism. Amoxicillin is a semi-synthetic penicillin that belongs to beta-lactams and is an inhibitor of peptidoglycan synthesis. It blocks penicillin-binding protein (PBP) transporters (13, 30). H. pylori becomes resistant to amoxicillin through mutations in the pbpa gene (PBP), the production of β-lactamases, and the presence of efflux pumps. The last two factors are associated with the highest resistance to this drug (25, 31).

The most frequent mutations in the pbpa gene substitutions are observed in specific positions: a) in amino acid 556, substitution from serine to threonine (Thr556→SER) takes place; b) in position 648, a substitution from lysine to a glycine (Lys648→Gly) occurs; c) in position 649, an arginine to lysine (Arg649→Lys) substitution, and d) in position 656, an arginine to proline (Arg656→Pro) substitution.

The first strains of *H. pylori* resistant to amoxicillin were recorded in 1997 in a patient who had previously been treated (32). In 2009, the appearance of a β-lactamases producing strain (TEM-1) capable of destroying the beta-lactamic ring of the antibiotic was described (25). In addition, efflux pumps were reported on the bacterial wall, expelling the antibiotic to the outside of the cell. Although this was only observed in in-vitro strains, it is of great concern as the pumps cause the strains to become multi-resistant (31).
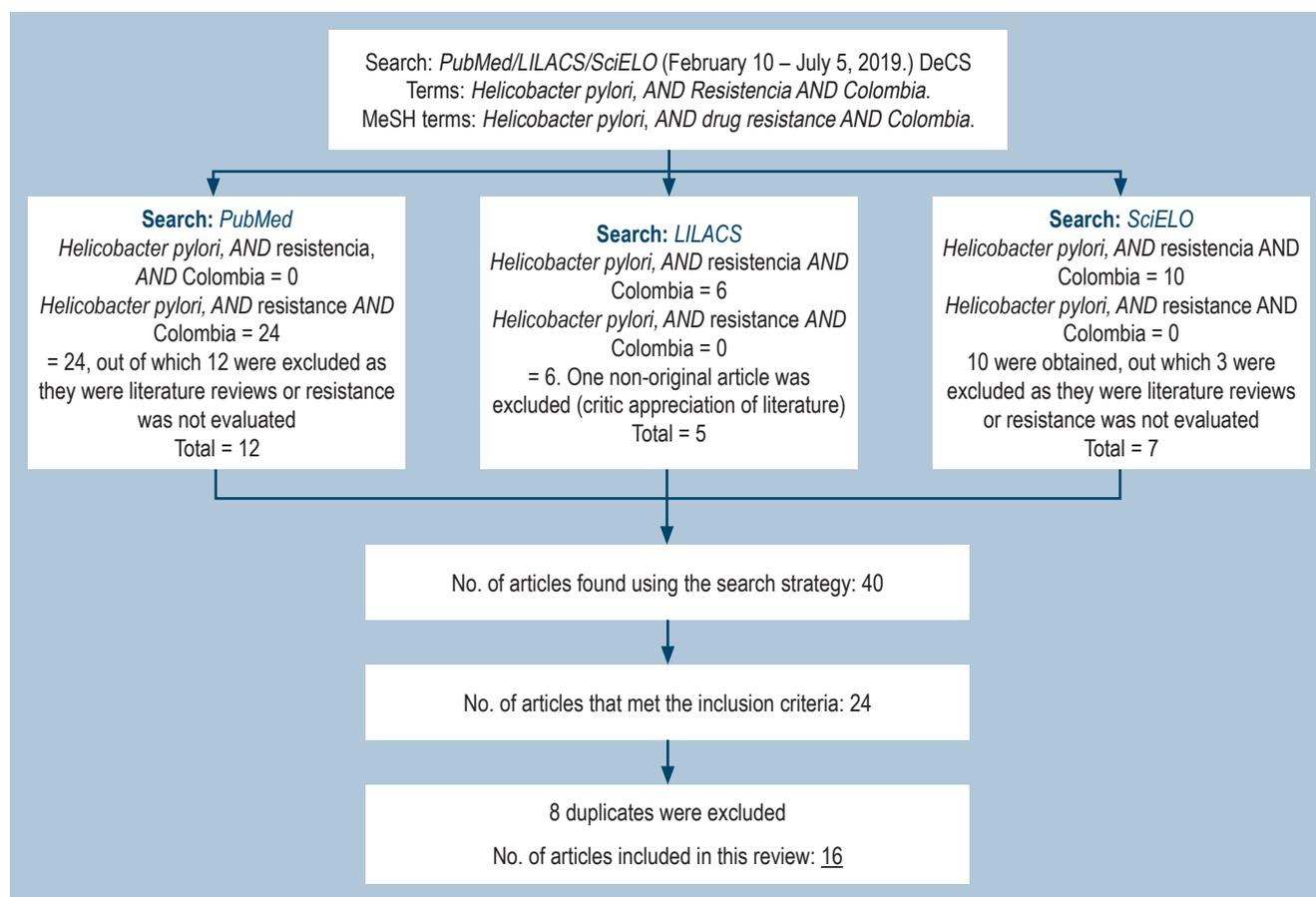
Figure 1. General outline of the review strategy on *Helicobacter pylori* antimicrobial resistance in Colombia. DeCS: Health Science Descriptors; MeSH: Medical Subject Headings.

Camargo et al., in a review conducted in 2014 and in which several studies on H. pylori resistance conducted in Latin America were included, found that Colombia and Brazil had the highest rates of amoxicillin resistance: 7 and 15%, respectively (12.) In addition, in 2007, Gómez et al., in a study were 648 physicians (68%: General physicians, 19%: Internist physicians, and 13%: Gastroenterologists) from 9 Colombian cities were surveyed, reported that amoxicillin was the most prescribed antibiotic to treat *H. pylori infection*, with a 73% rate (33). In Colombia, studies show *H. pylori* resistance rates to amoxicillin of 1.9%, 3.8%, and 9.5% (34-36).

Metronidazole

The bactericidal action of metronidazole depends on enzyme reduction. In the case of H. *pylori*, which does not have the superoxide dismutase enzyme, the decrease effect of metronidazole is achieved through NADPH (nicotina-mide adenine dinucleotide phosphate) nitroreductase and NAD(P)H flavin oxidoreductase to produce anionic radicals as nitrous derivatives and hydroxylamines, which lead to inhibition in the synthesis of nucleic acids when bound to the imidazole ring (37-39). The resistance mechanisms acquired by the bacteria point mainly to mutations in the RdxA gene, which encodes NADPH nitroreductase, and *FdxB*, which encodes ferredoxin (38, 39).

Some authors state that point mutations in *RdxA* and, less frequently, changes in *FrxA*. cause resistance to metronidazole Others claim that FrxA mutations only boost resistance caused by the inactivation of *RdxA*. Although mutations occur with *RdxA* function loss, not always such function loss implies a decrease in susceptibility to the drug, which leads to the fact that genotypic results obtained are not consistent with those *in-vitro* and *in-vivo results* (40.)

In addition, the agar dilution method and the epsilometer test (E-test) present inter- and intra-test variability and are not accurate, probably because of the environmental

Table 1. Summary Table with *H. pylori* resistance studies conducted in Colombia

Cities	Year	Test Used	Resistance Recorded in Percent (%)	Most Frequent Mutation	Recommendations	Authors
Pereira, Armenia, and Manizales	2009	E-test PCR	88%: metronidazole, 3.8 %: clarithromycin	A2143G (82 %) clarithromycin	Metronidazole should not be included in the empirical treatment of *H. pylori* infection in this region in the study population.	(30)
Pereira and Armenia	2009	E-test PCR	88%: metronidazole, 2.2 %: clarithromycin	A2143G clarithromycin	Metronidazole and clarithromycin are not recommended as the first line of treatment. 50% of the strains may have double resistance in the study population.	(31)
Bogotá	2008	Disk diffusion E-test	93%: metronidazole, 60%: clarithromycin, 86%: tetracycline, 7%: amoxicillin	Not evaluated	According to the Latin American consensus of *H. pylori* infections, treatment with metronidazole is not recommended in the study population.	(32)
Bogotá	2009	E-test Disk diffusion	72%: metronidazole, 15 %: clarithromycin	Not evaluated	Metronidazole is not recommended in the study population.	(33)
Bogotá	2010	E-test	81.01%: metronidazole, 17.72%: clarithromycin, 3.8%: amoxicillin	Not evaluated	Using metronidazole or clarithromycin is not recommended in triple therapy as a first-line treatment schedule in Bogotá. Resistance to metronidazole may lead to a 50% decrease in treatment effectiveness in triple and quadruple therapies.	(34, 35)
Bogotá	2013	Agar dilution PCR	13.6 %: clarithromycin	A2143G clarithromycin (90.5 %)	Clarithromycin is not recommended in triple therapy as a first-line treatment schedule in the study population.	(36)
Bogotá	2016	Agar dilution Direct sequencing	27.3%: levofloxacin	gyrA mutation N871	Resistance to levofloxacin has increased in recent years and has increased to the point that it should not be an acceptable choice for use in empirical treatment in Bogotá.	(37)
Bogotá	2018	PCR	8 %: clarithromycin	23SrDNA	Study conducted in children. Due to the resistance rate to clarithromycin is <15%, its use is recommended in conventional treatment in children.	(38)
Bogotá	2019	Agar dilution Direct sequencing	Data from previous studies in Bogotá 13.6%: clarithromycin, 81.01%: metronidazole, 27.3%: levofloxacin	gyrA Mutation N871	Metronidazole, clarithromycin, or levofloxacin are not recommended for the treatment of *H. pylori* infection in Bogotá. First record of multi-resistor isolates.	(16)
Bogotá	2019	PCR	38.1 %: clarithromycin	A2142G clarithromycin A2143G	High resistance to clarithromycin (>15%) suggests reconsidering its use in eradication treatments in the study population.	(39)
Antioquia	2019	PCR, RFLP	18.8%: clarithromycin	A2142G clarithromycin A2143G	First record of antibiotic resistance in Antioquia.	(40)
Tumaco	2012	Agar dilution	19.8%: clarithromycin, 20.5%: amoxicillin, 10.96% were resistant to both	Not evaluated	First record of multidrug strains resistant to clarithromycin and amoxicillin.	(41)
Túquerres	2015	Agar dilution	4%: clarithromycin, 2.7%: amoxicillin	Not evaluated	These antibiotics can be used freely based on the low resistance observed in the study populations.	(42)
Tumaco	2018	PCR	19.8%: clarithromycin	A2143G (69.8%), A2142G (11.7%) y A2142C (2.6%)	First record of multidrug strains resistant to clarithromycin and amoxicillin.	(43)
Popayán	2014	PCR Direct sequencing	4.3%: clarithromycin	A2143G clarithromycin	Resistance of *H. pylori* to clarithromycin in the study population suggests that standard triple therapy can be used empirically to eradicate the infection.	(44)
Popayán	2017	PCR Direct sequencing	No phenotypic aspects were evaluated	RdxA metronidazole (78%) D59N	The frequency of mutations in RdxA nitroreductase in *H. pylori* isolates in Popayán suggests that empirical treatments with metronidazole would not be a valid option as an eradication therapy in the study population.	(45)

E-test: Epsilometry test; PCR: Polymerase chain reaction; RFLP: Restriction Fragment Length Polymorphisms.

conditions necessary for the culture of H. pylori (39, 41.) Regarding the epsilometer test, some authors argue that it overestimates the resistance to metronidazole, and therefore propose to support the resistance diagnosis with molecular biology tests.

In Latin America, the reported resistance to metronidazole is >30%. However, in countries such as Peru and Colombia, the average documented resistance is 66 and 83%, respectively (12). In Colombia, different studies describe resistance rates to metronidazole, through microbiological methods, ranging from 72 to 97.6% (34-36). Also, in 2017, Acosta et al. evaluated the frequency of RdxA mutations by genotypic methods and found that 78.2% of cases (133/170) showed some genetic alteration associated with drug resistance (42). Metronidazole is one of the most prescribed antimicrobials by general practitioners in Colombia to treat *H. pylori infection*; thus, high resistance rates seem to be ignored (33).

Clarithromycin

Clarithromycin is a macrolide with bacteriostatic and bactericidal activity that inhibits protein synthesis by binding to the 23S component of the 50S ribosomal subunit (43). *H. pylori* becomes resistant to clarithromycin through mutations in the peptide-diltransferase region of the V domain of the HPrrnB23S gene, which encodes the 23S ribosomal RNA (43). The most frequent mutations in this gene are located at positions 2142, with the transition of an adenine to a guanine (A2142G) and the adenine–cytosine transversion (A2142C.) The adenine-to-guanine transition at position 2143 (A2143G) produces a conformal change in the antibiotic binding site and prevents its action (44, 45.) Other less frequent mutations exist, including T2182C (46), T2717C (47), G2224A, C2245T, and T2289C transitions (48).

In Latin America, a prevalence of macrolide resistance of 14% and 13% has been reported in Argentina and Mexico, respectively (12.) In Colombia, a study conducted in 2009 in the Coffee Growing Region showed low resistance to clarithromycin (2.2%). However, this antibiotic is used only to treat respiratory diseases in childhood, which would explain the difference between the percentages found (33, 35).

On the other hand, studies on the prevalence of macrolide resistance in Colombia describe values ranging from 13.6 to 63.1% (29, 34, 36, 49). As for genotypic resistance, it has been determined that the transition of adenine to guanine at position 2143 (A2143G) is the most frequent (90.5%), followed by the A2142G (7.1%) and A2142C (2.4%) transitions (29).

Furazolidone

Furazolidone is one of the nitrofurans proposed to treat *H. pylori* infection as salvage therapy. Nitrofurans are bacteriostatic, but at high doses they are bactericidal, and act similarly to nitroimidazoles. Little is known regarding the resistance mechanism to this group of antibiotics. It has been suggested that pyruvate-flavodoxin oxidoreductase (encoded by the gene *Por*CDAB) and 2-oxoglutarate oxidoreductase (*Oor*DABC) are the nitroreductases involved in this process (50).

Su et al. identified mutations associated with furazolidone resistance in the porD gene, with guanine-adenine transitions at position 353 (G353A), adenine-guanine at position 356 (A356G), and cytokine-thymine at position 357 (C357T), and in *oorD,* with adenine transitions (A041G, A122G) and transversion (C349A.) However, some drug-resistant strains did not carry mutations, so there is a possibility of other mutations that have not yet been determined (51). Evidence suggests that other enzymes would activate nitrofurans; however, there are few studies on this subject (52).

In Colombia, there is only one phenotypic resistance study on *H. pylori*, which in fact is a thesis, describing a resistance of 4.8%. Beyond that, there are no other studies on genotypic resistance to nitrofurans conducted in the country (33, 53) Therefore, the prescription of nitrofurans is very low and are recommended as a rescue therapy in countries such as Brazil, where a resistance of 3% has been reported, a similar figure to that found in Bogotá (12.)

Levofloxacin

Levofloxacin is a fluoroquinolone used in some cases as rescue therapy in unsuccessful treatments with clarithromycin. Fluoroquinolones act by binding to subunit A of DNA gyrase and prevent the formation of tetramer (two subunits A and two B), thereby blocking the function of this enzyme. Subunits A and B are coded by genes gyrA and gyrB, respectively (37). The resistance of *H. pylori* to fluoroquinolones is caused by mutations in *gyrA* and *gyrB,* and by alterations in porins.

In this way, mutations occur in the Quinolone Resistance Domain Region (QRDR) region of gyrA, which prevents antibiotic binding to gyrase, by altering the quinolone binding site in the DNA-gyrase DNA complex (54.) Mutations described as causing this phenomenon are unique mutations: $Asn_{87} \rightarrow Lys$, $Ala_{88} \rightarrow Val$, $Asp_{91} \rightarrow Gly$, Tyr or Asn, and double mutations in $Asp_{91} \rightarrow Asn$ and in $Ala_{88} \rightarrow Val$ (55).

In Colombia, a 2009-2014 follow-up study conducted in Bogotá that evaluated levofloxacin resistance in biopsies from patients who underwent endoscopy, found that resistance in 2009 was 11.8%, while in 2014 it was 27.3%. These authors considered that this increase is due to the use of this drug for treating respiratory and urinary tract infections (56).

Tetracyclines

Tetracyclines are used in quadruple therapy regimens for the eradication of *H. pylori* (13.) These have a bacteriostatic effect when they bind reversibly to the 30S ribosomal subunit and inhibit protein synthesis. The mutations described are found in the HPrrnA-16S gene, which codes for the 16S component of the minor subunit (30S.) The most common involve nucleotides 926-928 (AGA→TTC). A926T/A928C, A926G/G927T, A926G/A928C double mutations and point mutation at the A926G transition or the A939C transversion have also been described (57-61).

In studies conducted in Latin America, low tetracycline resistance rates have been reported, ranging from 6 to 14% (12.) However, for Colombia, Yepes et al. (2018) reported that 85.7% (72/84) of the strains isolated in their study showed resistance to this drug (34.) This data differs greatly from records in the rest of the continent.

DISCUSSION

According to the percentages of H. pylori resistance in Colombia, metronidazole stands out as the antibiotic that causes the greatest resistance in the bacteria (**Table 1**). In Bogotá, the recorded resistance values ranged from 72 to 93% (34, 36, 62), while in the Coffee Growing Region, it was 88% (35). The only genotypic study was conducted in Popayán, where the most frequent mutation was found in D59N (78%) (42). Different authors recommend avoiding using metronidazole to treat H. pylori infection in the cities where their studies were conducted. Due to the lack of more information, it is not possible to determine the situation in the rest of the country.

Clarithromycin is the antibiotic with the highest number of resistance studies conducted in Colombia, showing significant differences. In the case of Bogotá, resistance rates to this drug have varied considerably between studies: 13.6% and 60% (29, 34, 36, 62). In Tumaco, the resistance rate was 19.8% (63), while in Armenia and Pereira it was lower, 2.2% (35) and 3.8%, respectively (64).

In Popayán, the genotypic resistance to clarithromycin prevalence was 4.3%, and the A2143G transition was the most frequent (65), whereas in Antioquia it was 18.8%, where the frequency of the A2143G transition was 81.5% (49).

Similarly, resistance to amoxicillin was assessed in 3 studies, 2 of which were conducted in Bogotá. Resistance values were 7% in 2008 (34) and 3.8% in 2010 (36). The third study was carried out in Tumaco and found a resistance of 20.5% (63).

With regard to levofloxacin resistance values in Colombia, 11.8% of the strains studied in Bogotá during 2009 and 27.3% in 2014 were resistant to this drug (56). The authors of said study suggest that levofloxacin should not be considered as an antibiotic of choice for empirical therapy of H. pylori infection. Likewise, resistance to tetracycline was 85.7%, which is reported by the only study that has been developed in our country on the resistance of *H. pylori* to this drug (34).

On the other hand, two studies have shown the emergence of multidrug-resistant H. pylori strains. The first was carried out in Tumaco in 2012 and detected the presence of strains resistant to clarithromycin and amoxicillin (63), while the second, conducted by Arévalo et al. in 2019, described strains resistant to 2 or more antibiotics (amoxicillin, clarithromycin, levofloxacin and metronidazole) These strains were found in isolates obtained from patients with 3 or 4 failed treatments (16).

According to the clinical practice guidelines for the diagnosis and treatment of H. pylori infection in adults, in Colombia, avoiding the use triple combination therapy of amoxicillin, clarithromycin, and metronidazole or levofloxacin as first-line therapy is recommended, provided that evidence shows the primary resistance of H. pylori to this type of therapy. Therefore, local epidemiology should be the basis to suggest an adjusted and appropriate treatment and avoid using antibiotics empirically that may lead to therapeutic failure (14).

The data presented in this review show there are variations between populations, as well as the importance of determining the resistance pattern of each population in the country. This must be done in order to monitor the frequency in which the different resistances of the strains present in the country occur.

It is worth noting that the data obtained from the studies apply only to the study population and should not be extrapolated to the country as a whole. The following map is a graphical representation of the studies that were included in this review (**Figure 2**).

Thus, the findings of this review show the need for further studies to determine H. pylori resistance to antibiotics in the Colombian population in order to define the antibiotics that must be prescribed as first-line or rescue therapy in the country.

Funding sources
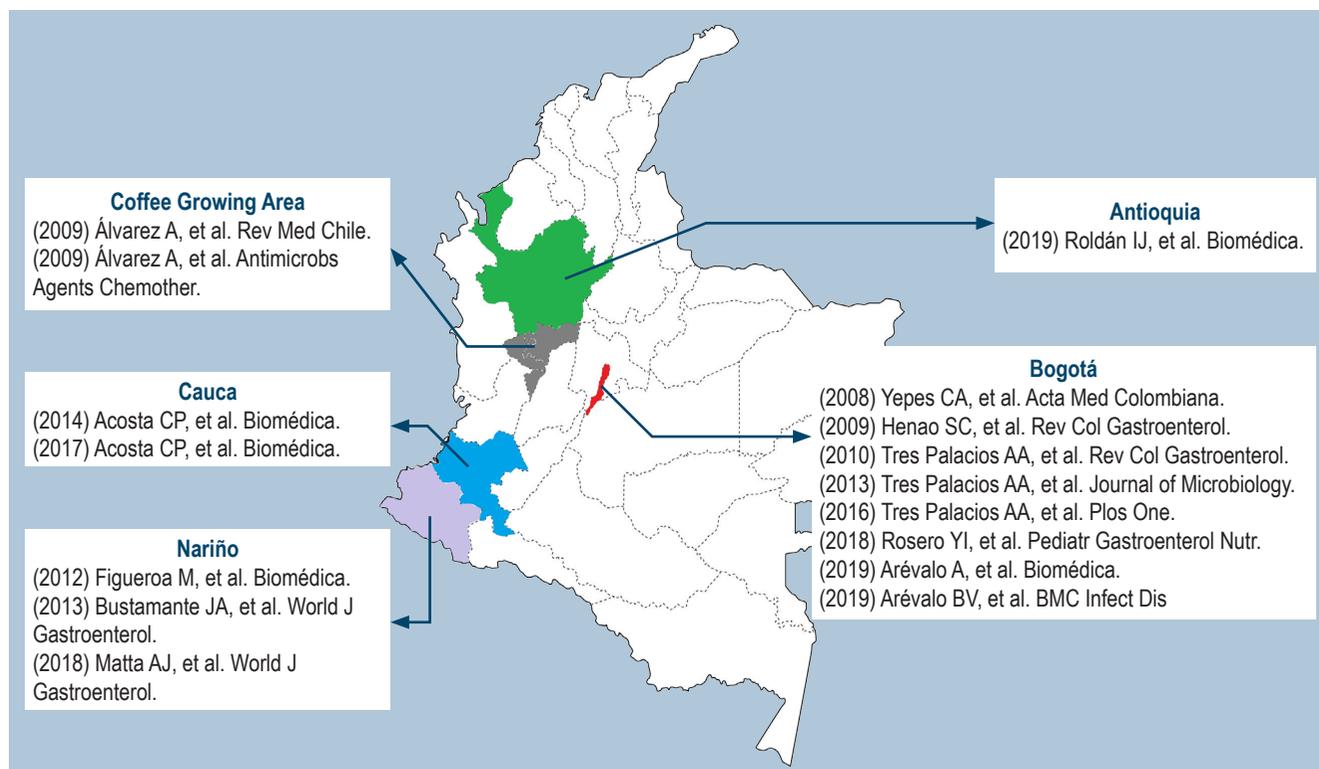
Figure 2. Map showing *H. pylori* resistance studies conducted in Colombia.

Within the figure:

Coffee Growing Area
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Antioquia
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Cauca
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Bogotá
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