

Susceptibility of Helicobacter Pylori to Antibiotics: a Study of Prevalence in Patients with Dyspepsia in Quito, Ecuador

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Abstract

Introduction: Worldwide, helicobacter pylori is associated with gastrointestinal pathologies, but increasing resistance to antibiotics used for its eradication is causing alarm. This study determined susceptibility of H. pylori to five antibiotics used in eradication therapy in an adult population with recurrent dyspepsia in Quito, Ecuador. **Materials and methods:** After patients provided informed consent, biopsies were taken from the gastric corpus and fundus of 210 patients with dyspepsia. H. pylori isolates identified by biochemical tests were recovered from cultures of biopsy samples. Minimal inhibitory concentrations (MIC) of metronidazole, clarithromycin, amoxicillin, tetracycline and levofloxacin were tested to indicate susceptibility. All cultures were correlated with the histopathological study. **Results:** H. pylori isolates were recovered from 89 cultures. A kappa of 0.9 was obtained between the culture and the histopathological study. The percentage of strains with antibiotic resistance were 63% for metronidazole, 66% for clarithromycin, 43% for amoxicillin, 36% for tetracycline and 54% for levofloxacin. **Conclusion:** These findings demonstrate high levels of resistance to the antibiotics used for eradication of H. pylori. Several factors including indiscriminate consumption of antibiotics and previous therapy may be involved.

Keywords

Helicobacter pylori; antibiotic resistance; minimal inhibitory concentration.

INTRODUCTION

Helicobacter pylori is a gram-negative bacillary bacterium that inhabits the gastric mucosa and is associated with inflammatory gastrointestinal diseases such as peptic ulcers, chronic gastritis and neoplasms such as MALT (mucosal-associated lymphoid tissue). Production of cytotoxins is associated with genes A (cagA) and the vacuolizer allele (vacA). (1, 2) H. pylori is acquired during childhood and remains asymptomatic for many years. Close contact is a risk factor. (3, 4) Studies have shown that the prevalence of H. pylori in adults in developing

countries exceeds 70%, (5) but in developed countries it ranges from 24% to 42%. (6)

H. pylori eradication therapy based on antibiotics associated with a proton pump inhibitor is up to 80% effective, but a percentage of therapeutic failure occurs and is mainly due to resistance to antibiotics. (7) An appropriate strategy for development of therapeutic guidelines based on local bacterial resistance data is the use of culturing and an antibiogram. (7, 8)

The study aims to determine the prevalence of antibiotic resistance in clinical isolates of H. pylori from patients older than 18 years who have recurrent dyspepsia.

MATERIALS AND METHODS

Patients

This study was carried out in two public specialty hospitals with approximately 400 beds in each in Quito, Ecuador over a period of 6 months. Patients over 18 years with symptoms of recurrent dyspepsia were selected. Those selected and who accepted signed an informed consent form and had not taken antibiotics for at least 5 days before upper gastrointestinal endoscopy.

Isolation and Identification of *H. pylori*

Two gastric biopsies were taken endoscopically: one from the fundus and one from the antrum. They were placed in a screw-cap bottle with 1 mL of sterile 0.9% saline solution and transported within two hours to the laboratory of the School of Bioanalysis of the Pontifical Catholic University of Ecuador (PUCE) or to the national reference laboratory of antimicrobial resistance of the National Institute of Public Health Research "Leopoldo Izquieta Pérez" (INSPI "LIP") for analysis. (9) After maceration, they were placed on Oxoid Columbia Agar Base supplemented with 7% lamb's blood with 10 mg/L vancomycin, 5 mg/L trimethoprim, 5 mg/L cefsulodin and 5 mg/L amphotericin B (DENT[®], Oxoid UK) following the manufacturer's instructions. They were incubated in a microaerophilic atmosphere with 5% oxygen (O₂), 10% carbon dioxide (CO₂) and 85% nitrogen (N₂) (CampyGen[®], Oxoid UK) at 37 °C for 7 days. Identification of *H. pylori* was done through morphological observation of the colony, gram staining (gram-negative bacilli), positive reaction for oxidase, catalase and urease tests. Isolates were stored in brain heart infusion (BHI) (Oxoid UK) with 20% glycerol at -70 °C.

Antimicrobial Susceptibility Tests

To reach McFarland Standard No. 3 for turbidity, a bacterial suspension was prepared from BHI broth (Oxoid UK) from a pure culture, incubated for 3 days in a microaerophilic atmosphere (CampyGen[®], Oxoid UK) at 37°C. (10) Later, it was inoculated on Mueller Hinton agar (Oxoid UK) supplemented with 7% lamb's blood. Gradient strips of minimum inhibitory concentration (MIC) were used for amoxicillin, metronidazole, tetracycline, levofloxacin and clarithromycin (Liofilchem, Italy) and then incubated for five days in a microaerophilic environment (CampyGen[®], Oxoid UK). The cut-off points for clarithromycin were taken from the Clinical and Laboratory Standards Institute (CLSI), while epidemiological cut-off points (ECV) were taken from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for the other antibiotics. *H. pylori* ATCC 43504 was used for quality control.

Ethical Issues

Prior to the study, all patients signed informed consent statements indicating the procedures that were to be carried out with the samples as well as the possible risks and benefits of participation in the study. The study was approved by the Bioethics Committee of each hospital and the PUCE.

RESULTS

Of the 210 people involved in the study, 143 had some degree of gastric inflammation and two people had adenocarcinoma. A total of 89 (42.4%) strains of *H. pylori* were isolated. Of these, 87 were found to have gastric pathologies by histopathological testing (Table 1). Four strains could not be recovered for susceptibility testing: metronidazole,

Table 1. Results of cytological study of gastric biopsies and number of *H. pylori* isolates recovered

Histological characteristics of gastric mucosa	Number of patients (%)	Number of <i>H. pylori</i> isolates recovered
Chronic non-atrophic gastritis	60 (28.6)	15
Chronic gastritis with intestinal metaplasia	28 (12.3)	15
Follicular chronic gastritis	23 (11)	23
Chronic diffuse gastritis	20 (9.5)	20
Chronic erosive gastritis	8 (8.8)	8
Gastritis crónica atrófica	4 (1.9)	4
Chronic atrophic gastritis	2 (1)	2
Negative	65 (26.9)	2
Total	210	89

levofloxacin, amoxicillin and tetracycline. Seventy-eight isolates were studied for clarithromycin. (Figures 1 and 2).

The largest number of strains that had MICs above the reference values were for metronidazole and clarithromycin, while the antibiotic association analysis for the different therapeutic schemes showed that 32% of the isolates were resistant to the combination amoxicillin/clarithromycin and 42% to clarithromycin/metronidazole (Table 2).

DISCUSSION

H. pylori is associated with inflammatory processes in the gastrointestinal mucosa and has prevalences of around 70% to 80% worldwide, especially in developing countries. (5) In our study, *H. pylori* was isolated in 89 patients (42.4%), only 2 isolates were not associated with gastric inflammation, and prevalence was lower than those reported in other studies. Factors such as the difficulty of isolating *H. pylori* from cultures, difficulties with biopsy sites and quality, transportation and incubation conditions can influence results. (11) In our study, the biopsies obtained from the fundus and gastric antrum were selected as recommended, (12) while culturing was done within 2 hours to avoid loss of viability of bacterial strains.

Both primary resistance associated with the use of antibiotics for the treatment of infections caused by pathogens other than *H. pylori* and secondary resistance caused by previous *H. pylori* eradication treatment were considered. (13-15) The CLSI establishes clinical cut-off points for clarithromycin, but does not do so for amoxicillin, tetracycline, metronidazole and levofloxacin. A variation among epidemiological studies has been observed regarding cut-off points. Our study used the epidemiological cut-off points given by EUCAST and used in most European countries, but we took into account both the possibility that isolates considered to be resistant can increase as well as demonstrated poor clinical efficacy. (16)

Although clarithromycin continues to be recommended as the first option for *H. pylori* eradication treatment, (8) our study shows that 66% of the isolates were resistant with values well above the 12% reported for Latin America, (17) and the 9.5% in Ecuador, reported in 2003 (18). Raymont et al. have demonstrated clarithromycin resistant isolates from 68% of patients with a history of prior antibiotic therapy, (19) and a similar percentage of resistance has been demonstrated by Gao et al. (20)

The Maastricht IV consensus on the use of macrolides recommends that local prevalence be taken into account for

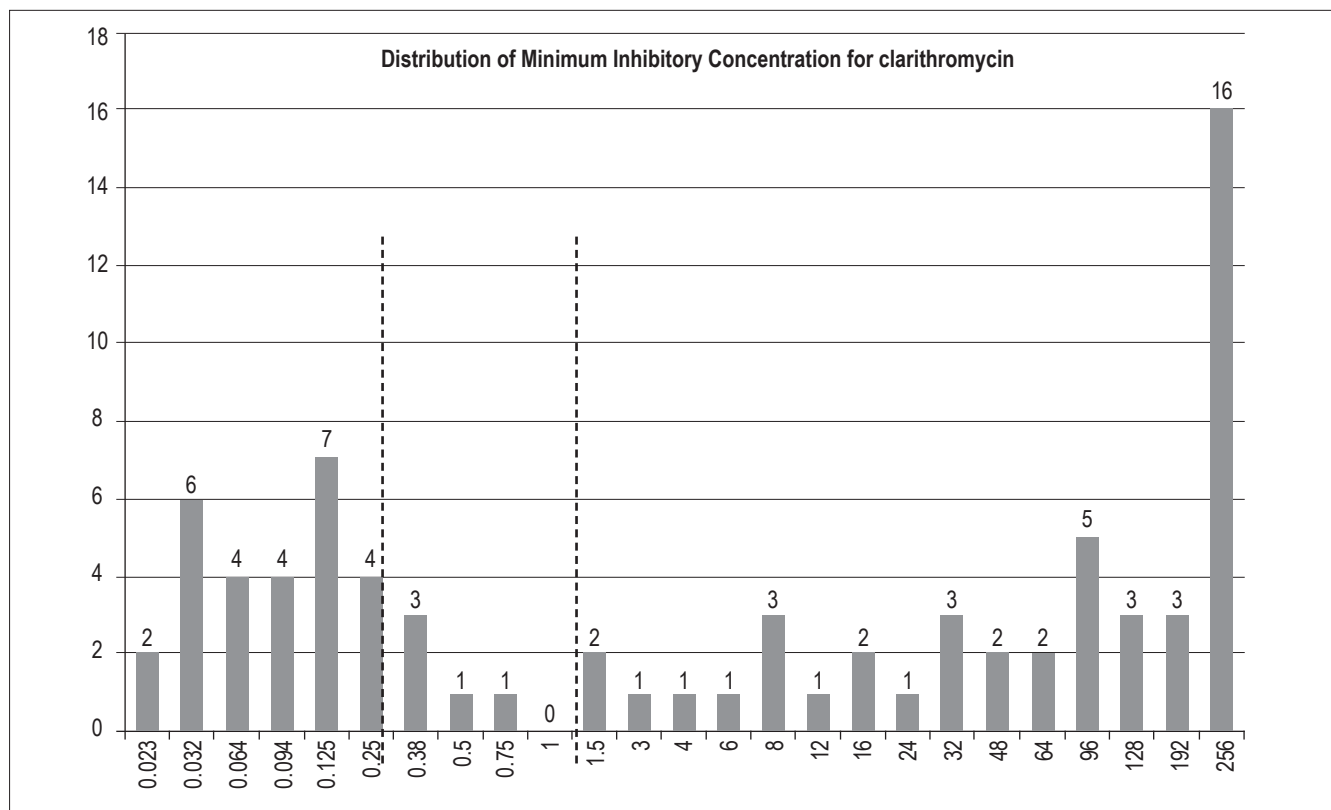


Figure 1. Minimum Inhibitory Concentration for clarithromycin in 78 strains of *H. pylori*. The broken lines represent cut points according to CLSI 2017.

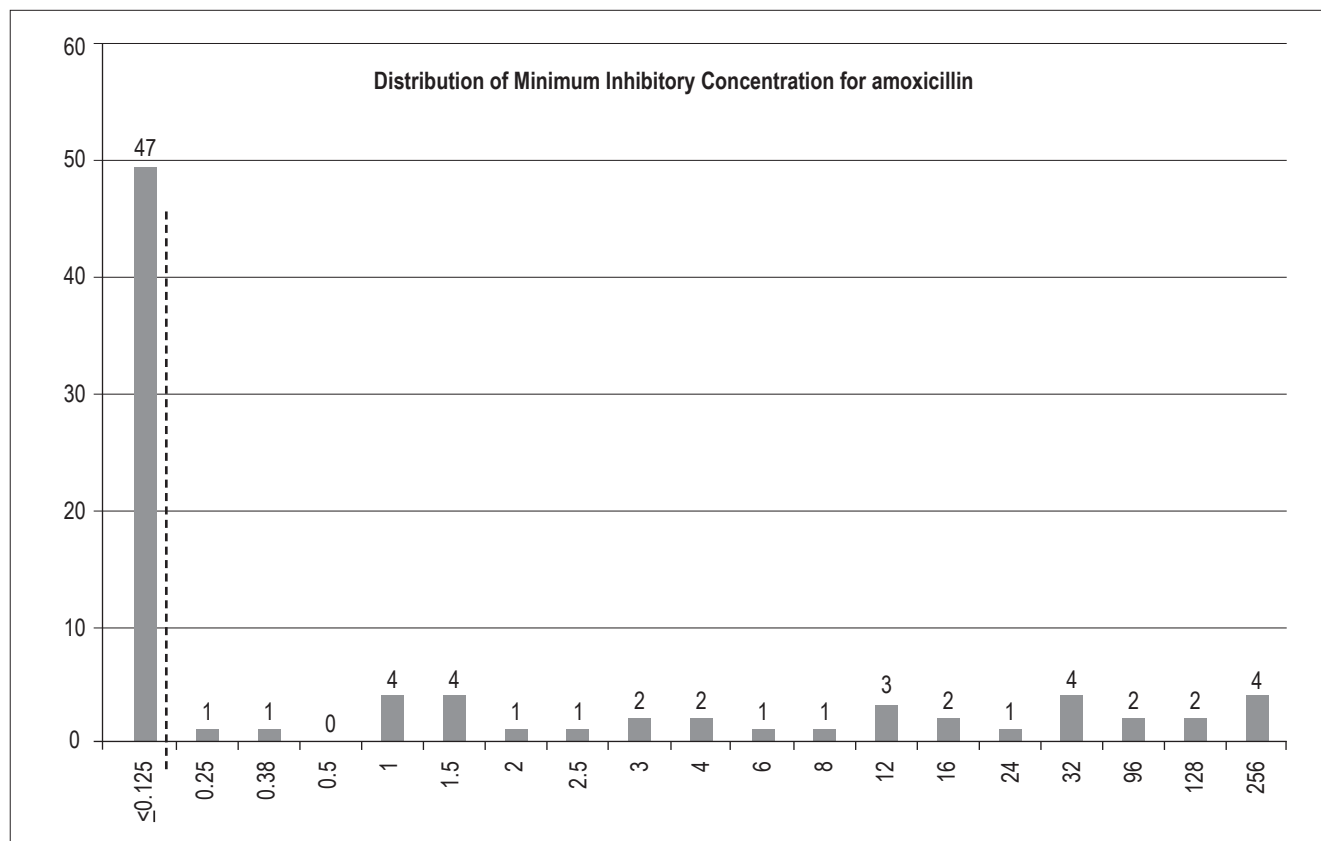


Figure 2. Minimum inhibitory concentration (MIC) for amoxicillin in 83 strains of *H. pylori*. The dashed lines represent epidemiological cut-off points according to EUCAST 2017.

Table 2. Percentage of resistance to antibiotics used in *H. pylori* eradication treatment. CLSI cut-off points for clarithromycin and EUCAST epidemiological cut-off points for other antibiotics.

Antibiotics Investigated	% of resistant <i>H. pylori</i> isolates according to ECV-EUCAST and CLSI
Levofloxacin	54
Tetracycline	36
Metronidazole	63
Clarithromycin	66
Amoxicillin	43
Combinations of antibiotics	Number and percentage of resistant <i>H. pylori</i> isolates
Amoxicillin/clarithromycin	26 (32)
Metronidazole/clarithromycin	32 (42)
Amoxicillin/levofloxacin	26 (32)

empirical treatment of *H. pylori*, and that clarithromycin's use should be avoided when susceptibility tests show 15% to 20% of resistant strains. (8) However, since these tests

are complex, few laboratories perform them. Among the mechanisms of resistance described for clarithromycin are mutations in the 23S rRNA gene and the use of proton pump inhibitors. (21, 22). A deficiency of our study was that we did not investigate these mechanisms.

Our study found that 43% of the strains of amoxicillin, another first-line antibiotic in triple therapy, were resistant with MICs greater than 0.125. This is a high percentage if one takes into account that for Latin America it is between 0% and 39%, and the difference is even greater for the percentage found in Ecuador which in previous years has not exceeded 4%. (17, 18) Nishizawa et al. have demonstrated that, similar to what has happened with the use of clarithromycin, after attempts to eradicate *H. pylori* with amoxicillin based schemes failed, the number of resistant isolates increased from 49.5% to 72%. (2, 3) In our study, we did not obtain data on previous administration of antibiotics within or outside of eradication therapy. Nevertheless, patients with recurrent dyspepsia were selected and may have received previous antimicrobial therapy. A resistance of 63% to metronidazole is within the range reported for Latin America of 12.5% to 95% and not dissimilar to the report from Ecuador of 80.9%. (17, 18)

More than 30% of strains present resistance simultaneously to two antibiotics used for eradication therapy, be it clarithromycin or levofloxacin.

Previous use of antibiotics is a risk factor for increasing resistance of *H. pylori* strains and, consequently, for therapeutic failure, either through primary or secondary resistance. (24) A high prevalence of resistance to antibiotics used in eradication treatment was demonstrated in this study, unlike the results obtained in Ecuador in 2003. Risk-benefit analysis together with the performance of susceptibility tests is recommended whenever clarithromycin or amoxicillin is used as an empirical treatment in patients with recurrent dyspepsia or in those who may have already received previous treatment.

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