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Antibiotic susceptibility in *Helicobacter pylori* strains isolated from Venezuelan patients with duodenal ulcer

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***Abstract*:** *Helicobacter pylori* antimicrobial drug resistance has appeared as an important cause of treatment failure. Samples were collected from 38 *H. pylori* infected patients with duodenal ulcers from the Gastroenterology Service of the "Hospital Vargas", Caracas, Venezuela (2009-2011) and were processed by the E-test method. The 94.8% isolates were sensitive to clarithromycin and erythromycin with MIC values of <0.5 mg/L and < 2 mg/L respectively. The 92.2% of the strains had a tetracycline MIC under 1 mg/L and 37% of the sensitive *H. pylori* strains had a metronidazole MIC of ≤8 mg/L. Metronidazole resistance occurred in 24/38 strains (63.1%), while resistance to erythromycin was found in 2/38 strains (5.2%), clarithromycin in 2/38 (5.2%), and tetracycline in 3/38 (7.8%) of the isolates. None of the strains was found to be resistant to amoxicillin. We observed 6/38 (15.8%) multi-drug resistance of *H. pylori* isolates in different combinations.

***Keywords*:** *Helicobacter pylori*, antibiotics, resistance, E-test.

Susceptibilidad a los antibióticos en cepas de *Helicobacter pylori* aisladas de pacientes venezolanos con ulcera duodenal

***Resumen*:** La resistencia de *Helicobacter pylori* a los antibióticos es una de las mayores causas de la falla terapéutica. Se usó el método de E-test en 38 cepas de *H. pylori* aisladas de pacientes con ulceras duodenales provenientes del Servicio de Gastroenterología, Hospital Vargas de Caracas, Venezuela (período 2009-2011). El 94,8% de las cepas fueron sensibles a claritromicina y eritromicina con una CMI de <0,5 mg/L y < 2 mg/L respectivamente. El 92,2% de las cepas fueron sensibles a tetraciclina (≤1 mg/L) y 37% de las cepas fueron sensibles a metronidazol con una CMI ≤8 mg/L. La resistencia a metronidazol ocurrió en 24/38 cepas (63,1%), eritromicina en 2/38 cepas (5,2%), claritromicina en 2/38 (5,2%), tetraciclina en 3/38 (7,8%) de los aislados. No se encontró resistencia a amoxicilina. Se observó 6/38 (15,8%) cepas multirresistentes en distintas combinaciones.

***Palabras clave*:** *Helicobacter pylori*, antibióticos, resistencia, E-test.

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Introduction

Helicobacter pylori infects the gastric antrum of half the world´s population and represents the major cause of acute and chronic gastroduodenal pathologies [1]. Several clinical trials have shown that bacterial eradication considerably reduces the long–term recurrences of peptic ulcers and that also induces a regression of primary low-grade B cell gastric lymphoma. Primary and acquired antimicrobial drug resistance has appeared as an important cause of treatment failure [2]. The prevalence of resistant strains and the establishment of the susceptibility levels to commonly used drugs are at least as important as the development of new antibiotics. At present, the most commonly used treatment is a triple therapy which is based on the association of an antisecretory drug (proton pump inhibitor (PPI) or H2 receptor antagonist) with two antibiotics: clarithromycin associated with amoxicillin or an imidazole derivative e.g. metronidazole or tinidazole [3].

The prevalence rate of metronidazole resistance has been found to vary appreciably between different populations and ethnic groups, from 10% up to 80% [4].

In Latin America, some countries have a high prevalence of *H. pylori* infection and associated diseases, particularly gastric cancer. This geographic region also has multiple avenues of unfettered access to antibiotics, including self-medication, unnecessary prescriptions, and lax sale regulations [4].

The aim of this study was to determine the antimicrobial drug resistances of *H. pylori* in clinical isolates from patients with duodenal ulcers who attended the Gastroenterology Service of "Hospital Vargas", Caracas, Venezuela (2009-2011 period).

Materials and methods

In this study 38 strains of *H. pylori* were isolated from patients with duodenal ulcers attended in the Gastroenterology Service of the "Hospital José María Vargas", Caracas, Venezuela (2009-2011 period). The gastric antrum samples obtained by upper digestive endoscopy were transported in physiological solution at 4 ºC and processed in a period of no more than 2 hours. The protocol was approved by the Bioethics Committee of the "Servicio Autónomo Instituto de Biomedicina Dr. Jacinto Convit" and an informed consent was obtained from all the participants.

A primary isolation was performed on Columbia agar with 10% human blood containing a supplement (Isovitalex™) in a microaerobic atmosphere (5% O_2, 10% CO_2, 85% N_2) for 5 days a 37 ºC. Prior to testing, the isolates were subcultured on Columbia agar with 10% horse blood at the same conditions of microaerobic atmosphere. Colonies from agar were scraped and suspended in 2 mL of Brucella broth.

The antimicrobial susceptibility of the *H. pylori* isolates was determined using the E-test (BioMerieux, Marci L'étoile France) and the plates were incubated for 72 h at 37 °C in microaerophilic atmosphere. Minimal inhibitory concentration (MIC) for resistance for each antibiotic was: amoxicillin (>0.12 mg/L), clarithromycin (>0.5 mg/L), erythromycin (>2 mg/L), tetracycline (>1 mg/L) and metronidazole (> 8 mg/L); according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [5].

Results

Table 1 shows the MIC values for the five antimicrobial agents investigated on the 38 *H. pylori* strains that were isolated in Caracas, Venezuela. Amoxicillin had <0.016 mg/L values of MIC for 100% of the strains. The MIC value of clarithromycin for 94.8% of the strains was <0.016 mg/L. For erythromycin, 94.8% of sensitive strains had a MIC value of <0.016 mg/L and 92.2% of the strains had a tetracycline MIC under this value. Only 37% of the *H. pylori* strains had a metronidazole MIC of <0.016 mg/L. Resistance to metronidazole occurred in 24/38 strains (63%; MIC >8 mg/L), while resistance to erythromycin occurred in 2/38 strains (5.2%; MIC ≥2 mg/L), clarithromycin in 2/38 (5.2%; MIC > 0.5 mg/L), and tetracycline in 3/38 (7.8% ; MIC >1 mg/L) of the isolated strains. None strains resistant to Amoxicillin were found.

Table 1. Antibiotic sensibility in *Helicobacter pylori* strains isolated from Venezuelan patients with duodenal ulcer. 2009-2011.

Antimicrobial agents	% Sensible strains	% Resistance strains	MIC (mg/L)
Amoxicillin	100*	0	> 0.12
Clarithromycin	94.8*	5.2	>0.5
Erythromycin	94.8*	5.2	>2
Metronidazole	37*	63	>8
Tetracycline	92.2*	7.8	> 1

*The MIC were <0.016 mg/L in all the cases.

In the study 6/38 (15.8%) *H. pylori* isolates were multi-drug resistance. Two strains were resistant to clarithromycin, tetracycline and metronidazole; two strains resistant to metronidazole and erythromycin; two strains resistant to tetracycline and metronidazole.

Discussion

The rational use of antibiotics is important for controlling emerging resistance; for this reason susceptibility testing of *H. pylori* must be included in the search for efficient antimicrobial combinations that could allow the eradication of this bacterium from the stomach.

In this study, we found that 63% of all *H. pylori* isolates were resistant to metronidazole. Resistance to metronidazole has been observed worldwide and presently occurs quite commonly in several countries. These results indicate a slight decrease in the resistance of *H. pylori* to metronidazole compared to previous studies in which antibiotic resistance was observed in 67% of strains [6]. After revision of the regional databases, evaluating *H. pylori* antibiotic resistance, Camargo *et al*. established summary prevalence's of antimicrobial, primary resistance among adults in Latin America of 12% for clarithromycin in 35 studies, 53% for metronidazole in 34 studies, 4% for amoxicillin in 28 studies, 6% for tetracycline in 20 studies and 8% for dual clarithromycin and metronidazole in 10 studies. Resistance prevalence varied significantly by country [4]. The global primary resistance rate for clarithromycin was 9.9% (95% confidence interval (CI) 8.3–11.7) [4]. The high frequency of metronidazole resistance observed in developing countries may be due to the fact that imidazole derivatives are used frequently for the therapy of intestinal parasite and gynecological infections.

Generally, antibiotic combinations are used for the control of *H. pylori* infections, but their indiscriminate use produces the emergence and spread of resistant strains. In the present study we found a frequency of multi-resistant *H. pylori* strains (15.8%), and metronidazole resistance

was always combined with clarithromycin or tetracycline resistance.

Macrolide resistance is based on defined point mutations in the peptidyl transferase loop in both copies of the 23S rRNA gene. Monotherapy with clarithromycin induces these mutations in up to 21% of patients infected with a susceptible *H. pylori* strain [7].

In regions of high (15%) dual clarithromycin and metronidazole resistance, bismuth containing quadruple therapies are the treatment of choice. Ideally, clarithromycin should be avoided and a combination of alternative antibiotics for which resistance does not becomes problematic should be used (eg, amoxicillin, tetracycline, furazolidone, rifabutin).

Currently, the triple therapy based on PPI plus clarithromycin (500 mg BID) and amoxicillin (1 g BID), for 10-14 days, is the first-line eradication scheme in Venezuela. However, some authors have reported a decrease in the effectiveness of this scheme; this has been attributed to the increase of resistance to clarithromycin [8].

Dib *et al.* had evaluated whether the eradication rate with triple therapy with levofloxacin is superior as first-line therapy to that with treatment using clarithromycin in the population that attended as outpatients at the Hospital of Lídice, Caracas, Venezuela. They found among the 42 patients in the control group, 14 eradication failures with 33.3% resistance to clarithromycin. In 39 patients in the experimental group, two eradication failures with 5.1% resistance to levofloxacin were observed. Triple therapy with levofloxacin can be implemented in populations where resistance to clarithromycin has been observed [9].

The main problem of *H. pylori* infection management is linked to antibiotic resistances. They are due to point mutations in bacterial DNA. The need for endoscopic procedure is the most important limit to their spread. There is increasing evidence of potential availability of noninvasive investigations able to detect *H. pylori* resistances to antibiotics, which may lead to tailored first-line therapies [10].

According to the results obtained in this study, amoxicillin may still be an antibiotic of choice for the treatment of infection by *H. pylori* in Venezuela.

It is clear that the emerging rates of antimicrobial resistance represent a significant challenge in the successful management of *H. pylori* infection and that resistance surveillance is warranted.

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Potential competing interest**:** The authors declare no conflict of interest.

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