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Antimicrobial Susceptibility and Mutations Involved in Clarithromycin Resistance in *Helicobacter pylori* Isolates from Patients in the Western Central Region of Colombia▽

Adalucy Álvarez,[1]* José Ignacio Moncayo,[1] Jorge Javier Santacruz,[1] Mario Santacoloma,[2] Luisa Fernanda Corredor,[1]† and Elizabeth Reinosa[1]

Universidad Tecnológica de Pereira, Centro de Biología Molecular y Biotecnología, Laboratorio de Microbiología y Parasitología, Pereira, Colombia,[1] and Universidad de Caldas, Departamento Clínico Quirúrgico, Manizales, Colombia[2]

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Resistance to metronidazole, clarithromycin, and amoxicillin (amoxicilline) was found in 82, 3.8, and 1.9% of 106 *Helicobacter pylori* isolates, respectively. No tetracycline-resistant isolates were found. In all of the clarithromycin-resistant isolates, only one point mutation was present, either A2143G or A2142G. Our results indicate that metronidazole should not be included in the empirical treatment of *H. pylori* infection in this region.

Helicobacter pylori is the major cause of chronic gastritis and peptic ulcer disease (21). The prevalence of *H. pylori* infection in dyspepsia patients in Colombia is higher than 80% (20).

Resistance to antimicrobials is of particular concern as a major cause of the failure to eradicate this pathogen (5, 23).

Treatment consists of triple therapy with a proton pump inhibitor plus two antibiotics (1, 27). In Colombia, the most commonly prescribed antibiotics are amoxicillin (amoxicilline), clarithromycin, and metronidazole (11).

Many reports have indicated that the prevalence of resistance varies geographically (16). In Colombia, only metronidazole resistance has been reported (11). The main reason for treatment failure has been *H. pylori* resistance to clarithromycin (16, 17). Monitoring of antimicrobial susceptibility patterns can provide general guidelines in the effort to eradicate *H. pylori* (6).

The usual phenotypic methods for susceptibility testing can be applied to *H. pylori* (3, 10). Numerous genotypic methods have been developed to detect clarithromycin resistance (16); the common methods used are PCR-restriction fragment length polymorphism (RFLP) and real-time PCR (17). Clarithromycin resistance is due to point mutations lying within the peptidyltransferase-encoding region of the 23S rRNA (4, 16, 21).

We conducted the present study to evaluate the prevalence of resistance to commonly used antimicrobial agents, the distribution of MICs, and the detection of the A2142G and A2143G mutations in *H. pylori* isolates from patients living in the western central region of Colombia.

A total of 106 *H. pylori* clinical isolates were obtained from gastric biopsy samples taken by endoscopy from patients with gastrointestinal symptoms in the western central region of Colombia (cities of Pereira, Armenia, and Manizales) who were part of a study to detect the prevalence of infection (unpublished data). The isolates were stored in Trypticase soy broth plus 20% glycerol at −80°C until used for MIC determination and detection of the A2143G and A2142G mutations.

Susceptibility to metronidazole, clarithromycin, amoxicillin, and tetracycline was determined by Etest according to the manufacturer's recommendations (EAS 013; AB Biodisk). The plates were incubated for 3 days at 37°C in a microaerobic atmosphere (5% O_2, 10% CO_2, and 85% N_2) in a TS Autoflow CO_2/O_2 water-jacketed incubator (NuAire). *H. pylori* ATCC 43504 was used as the control strain.

Chromosomal DNA was extracted from 48-h-old confluent cells with the Wizard genomic DNA purification kit (Promega) according to the manufacturer's recommendations. The point mutations of *H. pylori* were detected by a PCR assay that targeted a 267-bp 23S rRNA fragment with primers HPYS and HPYA (GenBank accession no. 881379) (18), followed by RFLP. The mutations A2143G and A2142G were detected with the BsaI and BbsI (New England BioLabs) restriction enzymes, respectively.

PCR amplification of DNA was performed in a Perkin-Elmer GeneAmp 9700 in a final volume of 50 μl as described by Ménard et al. (18). The 267-bp amplicon was analyzed by electrophoresis on a 3% low-melting-point agarose gel (Fisher), and bands were purified with Wizard PCR Preps (Promega). The purified amplicon was digested with BsaI for 1 h at 50°C and with BbsI for 1 h at 37°C. The restriction products were analyzed by electrophoresis on a 2.5% agarose gel. The amplicon was sequenced in all of the mutated isolates with the BigDye Terminador V3.1 kit (Applied Biosystems) in a ABI Prism 3100 genetic analyzer (Applied Biosystems).

All isolates were susceptible to tetracycline. Table 1 gives an overview of the distribution of the MICs and the number of resistant isolates.

The information on in vitro *H. pylori* susceptibility tests in Colombia is limited, except for one study reported in 1998

* Corresponding author. Mailing address: Universidad Tecnológica de Pereira, Facultad de Medicina, Laboratorio de Microbiología y Parasitología, Vereda La Julita, Pereira, Colombia. Phone: 057 6 3215393, ext. 15. Fax: 057 6 3215393, ext. 12. E-mail: adalucyalvarez@utp.edu.co.

† Present address: Fundación Universitaria del Área Andina, Carrera 24 No. 8-55, Pereira, Colombia.

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TABLE 1. Distribution of antibiotic MICs for 106 *H. pylori* isolates

Etest MIC range (mg/liter)	No. (%) of isolates resistant to:			
	Metronidazole	Clarithromycin	Amoxicillin	Tetracycline
<0.016	2 (1.9)	61 (57.5)	69 (65)	13 (12.3)
0.023–0.094	1 (0.9)	38 (35.8)	28 (26.4)	74 (69.8)
0.125–0.19	9 (8.5)	3 (2.8)	7 (6.6)	19 (17.9)
0.5–0.75	3 (2.8)			
1–2	3 (2.8)		2 (1.9)	
6–8	1 (0.9)			
16–32	8 (7.5)	3 (2.8)		
48–128	4 (3.8)			
>256	75 (70.8)	1 (0.9)		

(11). Our results confirmed the previous report on the high prevalence of metronidazole-resistant strains in our country and other developing countries (5, 7, 24). In those populations, metronidazole resistance in *H. pylori* is associated with the prior use of this drug to treat other bacterial infections; this could also be the case in Colombia (5, 11, 15, 17). Another important finding from our study was that for about 71% of the metronidazole-resistant isolates, the MICs exceeded 256 μg/ml; this has rarely been reported (14). The clarithromycin resistance in 3.8% of our isolates resembles the data from Chile and Paraguay but differs from those from Ecuador, Brazil, and Mexico (7, 9, 12, 19, 24). This low resistance could be due to low clarithromycin use, since this antibiotic is not a drug included in the Colombian National Health Program. The prevalence of *H. pylori* resistance to amoxicillin was very low, similar to previous reports (16, 17).

Both the Etest and PCR-RFLP detected the same four clarithromycin-resistant isolates, as has previously been reported (3, 10). Results obtained by PCR-RFLP help to specify the mechanism of *H. pylori* clarithromycin resistance, and this technique could also be applied directly to stool or biopsy samples (18, 22).

The four clarithromycin-resistant isolates harbor only either the A2142G mutation or the A2143G mutation. We found the A2143G mutation more frequently (in three-fourths of the isolates studied), as has previously been reported (2, 8). Amplicon sequencing showed that the mutations found by PCR-RFLP were present in both copies of the 23S rRNA in the four resistant isolates, and a mutation in one copy has been shown to be sufficient to confer clarithromycin resistance (13, 25). Several authors have shown that MIC levels vary according to the type of mutation (2, 26). Our study also found a high MIC with the A2142G mutation (MIC, >256 mg/liter).

In conclusion, we have determined, for the first time, *H. pylori* isolates resistant to metronidazole, clarithromycin, and amoxicillin from patients with gastrointestinal symptoms in the western central region of Colombia. The high prevalence of resistance to metronidazole precludes the use of this drug for the empirical treatment of *H. pylori* infection in this region. We believe that periodic monitoring of antimicrobial susceptibility is necessary to define the evolution of the resistance patterns of *H. pylori* in this geographical area and that a study on a larger scale should be carried out to determine the antibiotic resistance profile of *H. pylori* strains isolated from patients throughout the whole country.

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