vacA s1m1 genotype and cagA EPIYA-ABC pattern are predominant among Helicobacter pylori strains isolated from Mexican patients with chronic gastritis

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Abstract

Purpose. Virulent genotypes of Helicobacter pylori vacA s1m1/cagA+/babA2+ have been associated with severe gastric diseases. VacA, CagA and BabA are polymorphic proteins, and their association with the disease is allele-dependent. The aims of this work were: (i) to determine the prevalence of H. pylori by type of chronic gastritis; (ii) to describe the frequency of cagA, babA2 and vacA genotypes in strains from patients with different types of chronic gastritis; (iii) to characterize the variable region of cagA alleles.

Methodology. A total of 164 patients with chronic gastritis were studied. Altogether, 50 H. pylori strains were isolated, and the status of cagA, babA2 and vacA genotypes was examined by PCR. cagA EPIYA segment identification was performed using PCR and sequencing of cagA fragments of six randomly selected strains.

Results/Key findings. The overall prevalence of H. pylori was 30.5%. Eighty percent of the isolated strains were vacA s1m1, and the cagA and babA2 genes were detected in 74% and 32% of the strains, respectively. The most frequent genotypes were vacA s1m1/cagA+/babA2+ and vacA s1m1/cagA+/babA2−, with 40% (20/50) and 28% (14/50), respectively. In cagA+, the most frequent EPIYA motif was -ABC (78.4%), and EPIYA-ABCC and -ABCCC motifs were found in 10.8% of the strains. A modified EPIYT-B motif was found in 66.6% of the sequenced strains.

Conclusion. H. pylori strains carrying vacA s1m1, cagA+ and babA2+ genotypes were the most prevalent in patients with chronic gastritis from the south of Mexico. In the cagA+ strains, the EPIYA-ABC motif was the most common.

INTRODUCTION

Helicobacter pylori is a Gram-negative bacterium that colonizes the gastrointestinal tract of humans, mainly the gastric mucosa. These bacteria colonize around half of the world’s population, although their prevalence varies among geographical regions within a country, as well as between rural and urban areas, due to the socio-economic conditions, age and population ethnicity [1, 2]. Even though 80% of the infected population is asymptomatic, persistent infection by these bacteria causes chronic inflammation of the mucosa, which manifests as gastritis that can develop to chronic atrophic gastritis, intestinal metaplasia, dysplasia, and finally, gastric cancer [3–5]. Prevalence of infection by H. pylori and distribution of virulent strains, together with host factors, determine the regional variations in the incidence of gastric diseases [6].

Among H. pylori strains associated with severe gastric diseases are those that carry the babA2 and cagA genes, especially in combination with the genotype of vacA, s1m1 [7]. vacA encodes the protein VacA, a vacuolating cytotoxin secreted through the type V secretion system (T5SS) or autotransporter [8]. Besides forming pores in the cell membrane, this protein also induces apoptosis and inhibits cell proliferation and effector T-cell functions [9]. The vacA gene is present in all H. pylori strains. It has several isoforms, of which s1m1 is the most virulent, while the isoform s2m2 is the least virulent, and combinations of these result in isoforms of intermediate virulence [10]. CagA is
an effector protein encoded in the pathogenicity island cag-PAI. This protein is translocated through a type IV secretion system (T4SS) encoded in the same PAI, to the cytoplasm of the gastric cells where it is phosphorylated by Src and Ab1 kinases [11–13]. It is recognized as an oncoprotein because its phosphorylated form has affinity for proteins with phosphorylated tyrosine-binding domains, like tyrosine phosphatase Shp2. The interaction between CagA and its partners leads to the activation of signalling cascades involved in cell proliferation, apoptosis, cell cycle suppression and inflammatory responses in both phosphorylation-dependent and -independent manners [14]. The phosphorylation site of CagA is located on its C-terminal region, and comprises tyrosine residues that are part of a 5-amino acid sequence known as the EPIYA (Glu-Pro-Lle-Tyr-Ala) motif [12]. Four EPIYA motifs have been identified so far: EPIYA-A, -B, -C and -D, which are distinguished by the amino acid sequences that flank them. H. pylori strains that carry EPIYA-ABC motifs are found in Western countries, while those that carry EPIYA-ABD motifs are characteristic of Asian countries [15]. Western strains can produce variants of the CagA cytotoxin with up to five EPIYA-C motifs. Phosphorylation of CagA occurs mainly on the tyrosine residues of EPIYA-C and -D motifs, and the level of phosphorylation, as well as the carcinogenic potential, are related to a higher number of EPIYA-C motifs or to the presence of EPIYA-D [15–18]. Lastly, BabA is an afimbrial adhesin that binds Lewisb antigens in the gastric mucosa, thus inducing an autoimmune response to Lewis antigens, facilitating colonization, and increasing the response of IL-8 [19–21]. Its interaction with its receptor enhances CagA translocation, thus favouring the inflammatory response [22]. The bba gene has two isoforms babA1 and babA2, of which, babA2 encodes a functional protein [21].

The aims of this work were: (i) to determine the prevalence of H. pylori by type of chronic gastritis; (ii) to determine the frequency of cagA, babA2 and vacA genotypes in H. pylori strains isolated from Mexican patients diagnosed with different types of chronic gastritis; and (iii) to characterize the variable region of cagA alleles that encode the C-terminal region of CagA in order to determine the type and number of EPIYA motifs, as well as the frequency of their combinations.

**METHODS**

**Patients**

A cross sectional study was performed with 164 patients (61.6 % female, 38.4 % male) that were attended to at the Gastroenterology Service at the General Hospital ‘Dr Raymundo Abarca Alarcón’ and Specialized Unit in Gastroenterology Endoscopy, in Chilpancingo, Guerrero, Mexico. Patients were sequentially selected among those who attended for an endoscopic study due to dyspepsia symptoms. Only patients that had no H. pylori eradication treatment one month prior to the endoscopic procedure were selected. None of the patients included in this study were under treatment with proton pump inhibitors or with gastric pH neutralizing agents within 15 days prior to biopsy. Patients receiving non-steroidal anti-inflammatory therapy were excluded from the study. All patients signed a letter of consent. This project was approved by the Bioethics Committee of the Autonomous University of Guerrero, by the Department of Education and Research of the General Hospital ‘Dr Raymundo Abarca Alarcón’, and by the authorized personnel of the Specialized Unit in Gastroenterology Endoscopy.

**Biopsies**

The endoscopic study was performed after a fasting night with a video processor and video gastroscope (Fujinon, Wayne, NJ, USA). Two biopsies were taken from the antrum, one was immediately fixed in 10 % formalin for histological examination, and the other one was placed in Brain Heart Infusion Broth (BHI) (Becton Dickinson, NC, USA) with 10 % glycerol for the isolation of H. pylori. The biopsies were transported at 4 °C, and those intended for isolation of H. pylori were processed immediately.

**Histology**

Formalin-fixed biopsies were embedded in paraffin. Tissue sections of 4 µm were stained with hematoxylin-eosin for histological study. The histopathological diagnosis was carried out according to the updated Sydney system [23]. Endoscopic and histopathological findings were only used to diagnose patients.

**Isolation and identification of H. pylori**

Each biopsy transported in BHI broth with 10 % glycerol was macerated with a sterile wood applicator. In total, 50 µl of the homogenates were cultivated on Columbia Agar plates (Becton Dickinson, NC, USA) with 10 % ram blood, IsoVitaleX Enrichment and Helicobacter pylori selective supplement Dent (10 mg/L of vancomycin, 5 mg/L of trimethoprim, 5 mg/L of cefsulodin, 5 mg/L of amphotericin B) (Oxoid, Basingstoke, UK) at pH 6.8–7.0. The homogenates were distributed on the culture medium by isolation strip. The inoculated plates were incubated under microaerophilic conditions with 5 % O2, and 5 % CO2 at 37 °C in GasPak jars for 3–7 days. H. pylori was identified by colony morphology (small, transparent colonies, 1 mm in diameter), Gram staining and biochemical tests (urease, catalase and oxidase positive). H. pylori strain ATCC 43504 was used as a positive control.

**DNA purification**

Isolates identified as H. pylori were subcultured and incubated for 72 h. A pool of colonies from each biopsy was resuspended in extraction solution (10 mM Tris pH 8, 10 mM EDTA, 0.5 % SDS) for digestion with proteinase K. Total DNA was obtained by the phenol: chloroform: isoamyl alcohol technique [24]. Total DNA concentration was determined in a NanoDrop 2000 (NanoDrop Technologies, Wilmington, DE, USA). All DNA samples were stored at –20 °C until use.
Molecular confirmation and genotypification of vacA, cagA and babA2 of H. pylori strains

Confirmation of H. pylori strains was performed using oligonucleotides 16S1 and 16S2 (Table 1), which amplify a 522 bp fragment of the 16S rRNA, according to the method described by Román-Román et al. [25]. vacA genotyping and the status of cagA and babA2 were assessed by PCR with oligonucleotides specific for each region and gene (Table 1). The reaction mixture contained 1.5 mM MgCl₂, 0.2 mM dNTPs; 2.5 pmol of oligonucleotides F1 and B1, or 5 pmol of VAGF and VAGR, or 2.5 pmol of VAIF and VAIR, or 12.5 pmol of babA2F and babA2R; 1.5 U of Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA) and 200 ng of DNA, in a total volume of 25 µl. Amplification conditions were: one cycle at 94 °C for 10 min; 35 cycles at 94 °C for 1 min, 57 °C for 1 min, 72 °C for 1 min; and a final extension cycle at 72 °C for 10 min. The PCR products were subjected to 2.5% agarose gel electrophoresis, stained with ethidium bromide and visualized with ultraviolet light (UV). In each PCR, DNA from strain ATCC 43504 (vacAs1m1/cagA⁺/babA2⁺) was used as a positive control, and as a negative control, DNA was replaced with sterile deionized water. DNA from strain ATCC43504 (cagA⁺, cagPAI⁺) was used as a second negative control, and strain UEGE-644 (cagA⁺, cagPAI⁻) as a positive control. The presence of a 360 bp product was considered indicative of the absence of cagA and cagPAI [26, 27].

Detection of H. pylori CagA EPIYA motifs

Detection of CagA EPIYA motifs was performed by PCR with DNA from strains previously identified as cagA⁺. Four PCR reactions were performed per strain using antisense oligonucleotides cagA-P1C (EPIYA-A), cagA-P2TA (EPIYA-B), cagAWest (EPIYA-C) and cagAEast (EPIYA-D), and the cagA28F sense oligonucleotide (Table 1). Each reaction was carried out using 300 ng of DNA, following the conditions previously described [28]. As a positive control, DNA from strain ATCC 43504 (carrying EPIYA-ABCCC motif) was used, and as a negative control, the DNA was replaced with sterile deionized water.

Statistical analysis

The statistical program STATA v.12 was used for data analysis. Simple and relative frequencies of the qualitative variables were calculated, and Fisher’s exact test, X² test or Student’s t-test were used to determine differences between groups. A P-value <0.05 was considered significant.

Table 1. Oligonucleotides used in this work

| Gene                   | Oligonucleotide | Sequence                          | Size (bp) | Reference  |
|------------------------|-----------------|-----------------------------------|-----------|-----------|
| 16S rRNA               | 16S1            | 5'-GCTAAGAGATCAGGCTATGCTC-3'      | 522       | [50]      |
|                        | 16S2            | 5'-CTACGAGGTCAGTAAATGTC-3'        |           |           |
| vacA1, vacA2           | VAIF            | 5'-TGGGAATACAAACACAC-3'           | 259, 286  | [10]      |
|                        | VAIR            | 5'-TCGCTTGAATGGGCAAC-3'           |           |           |
| vacA1m1, vacA2m2       | VAGF            | 5'-CAATCTGCAATCAAGCGAG-3'         | 570, 645  | [51]      |
|                        | VAGR            | 5'-GCCTCTAATAATTTCAAGG-3'         |           |           |
| cagA                   | F1              | 5'-GATAACAGGGAAAGTTTGGCGA-3'      | 349       | [45]      |
|                        | B1              | 5'-CTGCAAAAGGTTTTGTCAGA-3'        |           |           |
| babA2                  | bab2F           | 5'-ATCCAAAAGAGGAAACATGAA-3'       | 850       | [20]      |
|                        | bab2R           | 5'-TGTTAGTAGATCCTGCGGAGACA-3'     |           |           |
| EPIYA                  | cagA28F         | 5'-TTTCTAAGGGACATTGGC-3'          |           | [52]      |
| -A                     | cagA-P1C        | 5'-GTCCTGCTTTTTTATTTAATTTAGC-3'  | 264       |           |
| -B                     | cagA-P2TA       | 5'-TTTAGCAACTTGATAAATGGG-3'       | 306       |           |
| -C                     | CagAWest        | 5'-TTTCAAAGGGAAAGTCCGGC-3'        | 501       |           |
| -D                     | CagAEast        | 5'-AGAGGGAGAGCTCCTTGATT-3'        | 495       |           |
| Empty site             | ESf             | 5'-CATGCGAGCGCGATGTG-3'           | 360       | [27]      |
|                        | ESr             | 5'-CATGCGAGCGCGATGTG-3'           |           |           |
RESULTS

Patients

We analysed 164 patients with histopathological diagnosis of chronic gastritis, of which 89.6 % (147/164) showed \textit{H. pylori}-associated chronic gastritis. Of these, 51 % (75/147) had chronic superficial gastritis, 25.2 % (37/147) had active chronic gastritis, and 23.8 % (35/147) had follicular chronic gastritis, while the rest of the patients (10.4 %, 17/147) showed reactive gastritis (Table 2). The mean age of the patients was 48±17 years old (ranging between 19–89 years). In total, 61.6 % (101/164) were female patients, and 34.8 % (57/164) had undergraduate or higher education.

All strains identified as 35.3 % (6/17) with reactive gastritis were gene by PCR (Fig. 1a). Regarding the types of gastritis, 60 % (90/150) of the patients with chronic active gastritis, and 34.8 % (57/164) had undergraduate or higher education.

The frequency of \textit{H. pylori} isolation was 30.5 % (50/164). No significant differences were found between age, gender or schooling level of \textit{H. pylori} positive and negative patients (Table 2). All strains identified as \textit{H. pylori} by culture were confirmed by amplification of a fragment of the 16S rRNA gene by PCR (Fig. 1a). Regarding the types of gastritis, 60 % (21/35) of the patients with chronic follicular gastritis, and 35.3 % (6/17) with reactive gastritis were \textit{H. pylori} positive, while those with chronic active gastritis and chronic superficial gastritis showed a lower infection frequency, 24.3 % (9/37) and 18.7 % (14/75), respectively. The frequency of infection was significantly different among diagnoses (P<0.001).

Genotyping of \textit{vacA}, \textit{cagA} and \textit{babA2} status

In order to determine the \textit{vacA} genotype and the status of \textit{cagA} and \textit{babA2}, DNA of all isolates was subjected to PCR. Overall, 80 % (40/50) of the isolates harboured the \textit{s1m1} allelic variant of \textit{vacA}; 74 % (37/50) carried \textit{cagA} and 32 % (16/50) were \textit{babA2} positive, with 40 % (20/50) and 28 % (14/50), respectively. Alгоgether, 70 % (35/50) of the isolates were \textit{vacA s1m1/cagA+}, and of these, 40 % (14/35) were \textit{babA2+}. Of all the patients analysed, only one showed mixed infection with two strains carrying different \textit{vacA} genotypes (\textit{s1m1} and \textit{s1m2}), both \textit{cagA/babA2}. Of the strains with \textit{vacA s1m1/cagA+} genotype, 54.3 % (19/35) was isolated from female patients. No significant difference was found between \textit{H. pylori} genotype and patient gender.

However, when we analysed the frequency of \textit{H. pylori} according to the types of chronic gastritis, we found that there was a significant difference (Table 4), the absence of the bacterium being more prevalent, except in follicular chronic gastritis where 60 % (21/35) of the cases were \textit{H. pylori} positive. Regarding the genotypes, \textit{vacA s1m1} isolates were the most frequent in all types of gastritis, including reactive gastritis (83 %, 5/6), while \textit{cagA} positive strains predominated in all groups, with frequencies that varied between 55.6 and 85.7 %. The highest frequency of isolates was \textit{babA2} negative in all types of gastritis, and the highest percentage of strains \textit{babA2} positive were isolated from patients with follicular chronic gastritis (35.7 %, 5/14).

\textbf{cagPAI negative clinical isolates}

To corroborate the absence of \textit{cagPAI} in the 13 \textit{H. pylori} \textit{cagA+} strains, DNA of the isolates was subjected to the conventional PCR empty site assay. In 100 % (13/13) of \textit{cagA} isolates, a 360 bp fragment was amplified (Fig. 1b), corroborating that 13 of the 50 patients (26 %) with chronic gastritis harboured \textit{H. pylori} strains lacking \textit{cagPAI}.

\textbf{Polymorphisms of \textit{cagA} in EPIYA sequences}

To determine the type of EPIYA motifs of \textit{H. pylori} \textit{cagA+} isolates, conventional PCR was performed using primers listed in Table 1. Six electrophoretic patterns were observed in the amplified fragments, corresponding to the combinations of EPIYA motifs: AB, ABC, ABCC, ABCCC, AABCC and AABCC. Of the 37 isolates, 2.7 % (1/37) contained two motifs (AB), 78.4 % (29/37) contained three (ABC), 10.8 % (4/37) contained four (ABCC and AABCC), and 8.1 % (3/37) contained five (ABCCC and AABCC) (Fig. 2a). All the isolates with three motifs had the combination ABC. Four strains (10.8 %) carried two EPIYA-C motifs, three had the ABC combination, and the fourth had two A sequences (Table 5). In two male patients, \textit{H. pylori} \textit{cagA+} with EPIYA-ABCCC motifs was isolated. With respect to the gastric pathology, two isolates with EPIYA-ABC combination were obtained from patients with follicular chronic gastritis, and one from superficial chronic gastritis. One strain with the EPIYA-ABCCC motif was isolated from a patient with reactive gastritis, and one from a patient with follicular chronic gastritis. The only strain with EPIYA-AABCC was isolated from a patient with superficial chronic gastritis. As expected, we found no strains with the EPIYA-D motif. These results

### Table 2. Socio-demographic characteristics, \textit{H. pylori} infection and histopathological diagnosis of 164 patients with chronic gastritis

| Chronic gastritis | \textit{H. pylori} negative | \textit{H. pylori} positive | P-value |
|-------------------|---------------------------|--------------------------|--------|
| Age (years; mean±SD) | 49±16 | 48±18 | 0.609* |
| Gender, n (%) | | | |
| Female | 75 (65.8) | 26 (52) | 0.117† |
| Male | 39 (34.2) | 24 (48) | |
| Schooling, n (%) | | | |
| Unschool | 9 (7.9) | 3 (6) | 0.190‡ |
| Elementary | 28 (24.5) | 13 (26) | |
| Junior high | 14 (12.3) | 13 (26) | |
| High school | 22 (19.3) | 5 (10) | |
| Undergrad or higher | 41 (35) | 16 (32) | |
| Types of chronic gastritis, n (%) | | | |
| Superficial chronic gastritis | 61 (53.5) | 14 (28) | <0.001 |
| Active chronic gastritis | 28 (24.6) | 9 (18) | |
| Follicular chronic gastritis | 14 (12.3) | 21 (42) | |
| Reactive gastritis | 11 (9.6) | 6 (12) | |

*Student’s t-test.  †Χ² test.  ‡Fisher’s exact test.
were confirmed by sequencing the ~650 to ~850 bp fragment of the 3′ variable region of cagA of six randomly selected strains. The chosen strains contained EPIYA motifs -ABC (strains UEGE666, UEGE696 and HG162), -ABCC (strain HG193) and -ABCCC (strains UEGE846 and UEGE751), as well as the vacA variant s1m1. Unexpectedly, only two EPIYA-C were detected in sequences of strains UEGE846 and UEGE751. Besides corroborating that our isolates belonged to the Western type, sequencing results showed that 66.7% (4/6) of the strains carried the variant EPIYT-B motif (Fig. 3). This variant was found in two strains containing the -ABC motif (UEGE666, UEGE696), one with the -ABCC motif (HG193) and one with the -ABCCC motif (UEGE751) (Table 4). These four strains had the genotype vacA s1m1/cagA+/babA2+.

**DISCUSSION**

*H. pylori* infection is associated with the development of gastric pathologies, and the frequency of infection varies among regions. In the present study, *H. pylori* was isolated with a frequency of 30.5% (50/164), which is lower than that reported by Paniagua et al. in 2009 (60.1%) in Mexican patients with chronic gastritis [29], but higher than those reported by Chihu et al. in 2005 (16.7%) [30] in patients with chronic active gastritis, and Lopez-Vidal et al. in 2008 (26%) [31] in patients with non-cancerous gastric diseases. These differences show the variability of *H. pylori* distribution in different regions of Mexico.

The frequency of *H. pylori* was significantly different between the types of chronic gastritis (*P*<0.001). The prevalence of infection in cases of reactive gastritis (35.3%) was higher than in cases of superficial chronic gastritis (18.7%) and active chronic gastritis (24.3%), but lower than in cases of follicular chronic gastritis (60%). The frequency of *H. pylori* in reactive gastritis is similar to that reported for Chilean patients (33%) [32], but it contrasts with that found in Colombian patients (18.5%) [33]. These differences can be explained by the geographic origin, the genetic background and the socio-demographic characteristics of the populations. In this research we analysed patients that attended the Gastroenterology Service at the General Hospital ‘Dr Raymundo Abarca Alarcón’ and the Specialized Unit

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**Table 3.** Distribution of vacA/cagA/babA2 genotypes of *H. pylori* strains per gender of patients with chronic gastritis

| Genotypes | Gender |   |   | P-value |
|-----------|--------|---|---|---------|
|           | Female | Male |   |        |
| vacA s1m1/cagA+/babA2+ | 6 (23) | 8 (33) | 0.481* |
| vacA s1m1/cagA+/babA2- | 12 (48) | 8 (33) |   |
| vacA s1m1/cagA+/babA2- | 1 (4) | 3 (13) |   |
| vacA s2m2/cagA+/babA2+ | 1 (4) | 0 |   |
| vacA s2m2/cagA+/babA2- | 5 (19) | 3 (13) |   |
| vacA s1m1 and s1m2/cagA+/babA2+ | 0 | 1 (4) |   |
| vacA s2m2/cagA+/babA2- | 1 (4) | 0 |   |
| vacA s2m2/cagA+/babA2- | 0 | 1 (4) |   |

*Fisher’s exact test.
Evidence indicates that the isoform number of viable bacteria on the tissue mucosa, to the site where the biopsy was taken, and to the may be due to the distribution of the bacteria on the gastric ces in the frequency of etiological agents [33, 34]. On the other hand, the differen-
ting a gastric ulcer due to the synergic effect of the mixed etiology in these patients, which have a higher risk of H. pylori-
were from patients with reactive gastritis, a type of non-
patients with this type of gastritis, while only 12 % (6/50) majori-
of the isolates (42 %, 21/50) were obtained from of gastritis except follicular chronic gastritis. Actually, the socio-economic conditions since it is a particular clinic.

| Types of chronic gastritis | H. pylori-associated | Non-H. pylori- associated |
|---------------------------|----------------------|--------------------------|
| H. pylori                  | n=75                 | n=37                     | n=35                     | n=17                     |
| Positive                  | 14 (18.7)            | 9 (24.3)                 | 21 (60)                  | 6 (35.3)                 |
| Negative                  | 61 (81.3)            | 28 (75.7)                | 14 (40)                  | 11 (64.7)                |
| vacA genotypes            |                      |                          |                          |
| s2m2                      | 1 (7.1)              | 3 (33.3)                 | 4 (19.1)                 | 1 (16.7)                 |
| s1m2                      | –                    | –                        | 1 (4.8)                  | –                        |
| s1m1                      | 13 (92.9)            | 5 (55.6)                 | 16 (76.1)                | 5 (83.3)                 |
| s1m1/s1m2                 | –                    | 1 (11.1)                 | –                        | –                        |
| cagA                      | Positive             | 12 (85.7)                | 5 (55.6)                 | 16 (76.2)                |
| Negative                  | 2 (14.3)             | 4 (44.4)                 | 5 (23.8)                 | 2 (33.3)                 |
| babA2                     |                      |                          |                          |
| Positive                  | 5 (35.7)             | 2 (22.2)                 | 7 (33.3)                 | 2 (33.3)                 |
| Negative                  | 9 (64.3)             | 7 (77.8)                 | 14 (66.7)                | 4 (66.7)                 |

*Fishier’s exact test.

in Gastroenterology Endoscopy; the first one serves people with very low socio-economic level and no access to social security services, while the latter attends people with better socio-economic conditions since it is a particular clinic.

H. pylori negative samples were more prevalent in all types of gastritis except follicular chronic gastritis. Actually, the majority of the isolates (42 %, 21/50) were obtained from patients with this type of gastritis, while only 12 % (6/50) were from patients with reactive gastritis, a type of non-

The presence of babA2, which encodes the H. pylori active protein BabA, has been associated with peptic ulcer disease and gastric cancer in Western countries [41]. In this work, the frequency of babA2+ was 32 %, which is higher than that reported in 2009 for Mexican patients with chronic gastritis [29], but lower than that reported from isolated strains from pediatric patients between 10 months and 17 years old from Mexico City [42]. We are unable to rule out the possibility that the frequency of babA2+ found in this work was not influenced by the existence of babA2 allelic variants not detected with the primers used. Of the babA2+ strains, 93.7 % (15/16) carried the vacA s1m1 allele. Our results showed that the vacA s1m1/cagA+/babA2+ genotype was found in 28 % of the isolated strains. This frequency is lower than that found in Mexican pediatric patients (47.5 %) [42]. VacA and BabA proteins produced by vacA s1m1/babA2+ strains have a synergistic effect on H. pylori virulence, increasing the risk of a severe gastric disease [35].

cagA+ strains were found in 74 % of the isolated strains, a frequency similar to that found by Reyes-Leon et al. in 2008 (78.6 %) in strains from pediatric patients with chronic abdominal pain and adults with non-ulcerous dyspepsia or peptic ulcer [43], but higher than that found by Paniagua et al. in 2009 (52.4 %) by multiplex PCR [29]. In pediatric patients from Mexico City the frequency of cagA+ strains has reached 90.6 % [42]. In strains from Colombian patients with diverse gastric pathologies, the frequency of cagA+ strains is up to 83.8 % [44]. The presence of cagA was found more frequently along with the vacA s1m1 variant (70 %), which is consistent with previous reports [45–47].
The most frequent EPIYA motif found in our isolated strains was -ABC (78.4%), which is in agreement with previous reports from Mexican patients with gastric disease by Beltran-Anaya et al. and Rizzato et al. [28, 48]. This motif was found in a lower proportion (50%) in Mexican pediatric patients [42]. Only two of the 37 isolated strains (5.4%) carried the EPIYA-ABCCC motif, a proportion that is lower than that reported by Mendoza-Elizalde et al. in 2015 (18.75%) [42], but higher than that found by Rizzato et al. in 2012 (3.7%) in strains from Mexican and Venezuelan patients with chronic gastritis [48]. In 18.9% of the isolated strains the number of EPIYA motifs was ≥4, and of these, 16.2% had two or three EPIYA-C motifs. In the two isolations with three EPIYA-C by PCR the third C motif could not be found by sequencing. The reason for this is not clear. Four out of six sequenced strains had a modified EPIYT-B motif, which is the most frequent EPIYA-B alternative in Western strains [49]. It has been shown that the EPIYT-B motif is associated

Fig. 2. Characterization of EPIYA motifs. (a) Representative gel electrophoresis of PCR products of EPIYA motifs of strains: HG-162 (EPIYA-ABC), UEGE-751 (EPIYA-ABCCC) and HG-193 (EPIYA-ABCC). Strain ATCC43504 (EPIYA-ABCCC) was used as a positive control. Lanes: MW, 100 bp molecular weight marker; A, EPIYA-A motif (~256 bp); B, EPIYA-B motif (~306 bp); and C, EPIYA-C motif (first, 501 pb; second, ~650 pb; and third, >650 bp). (b) Frequency of CagA EPIYA motifs of H. pylori strains from patients with chronic gastritis.

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with duodenal ulcers, and with induction of lower levels of cellular elongation and IL-8 secretion [43, 49]. This modified motif has been found before in Mexico in two different regions, being more prevalent in the state of Guerrero [28, 42].

It is probable that in this geographic region of Mexico, strains with the modified EPIYT-B motif are the most frequent, although this should be addressed in more detail.

Patients infected with *H. pylori* cagA+ strains that carry more than one EPIYA-C motif have a higher risk of developing atrophic gastritis and gastric carcinoma, since CagA possesses more C-terminal phosphorylation sites, a characteristic associated with a higher carcinogenic potential [49]. However, it is possible that this effect could be attenuated by the EPIYT modification in the EPIYA-B motif, and that this CagA variant attenuates the pathogenic effect caused by the three or more EPIYA-C motifs in patients infected with *vacA s1m1/babA2* strains.

In conclusion, our results document an important diversity of Western variants of cagA in *H. pylori* strains isolated from patients with chronic gastritis. The prevalence of *H. pylori* is significantly different between the different types of chronic gastritis, and in these, the genotypes *vacA s1m1/...
cagA⁺ are the most prevalent. The 3’ variable region of cagA, and thus the CagA protein of H. pylori strains from the south of Mexico is heterogeneous in the number and type of EPIYA motifs. In these H. pylori isolates, the vacA s1m1 genotype along with cagA variants encoding EPIYA-ABC patterns were predominant, and a significant proportion of these were babA2⁺. H. pylori strains containing the EPIYT-B motif in combination with one or more C motifs are common in patients with chronic gastritis.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
This project was approved by the Bioethics Committee of the Autonomous University of Guerrero, and by the Department of Teaching and Research of the General Hospital ‘Dr Raymundo Abarca Alarcón’ in Chilpancingo City. Patients signed informed consent statements. All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee, and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

References
1. Carter FP, Frankson T, Pintard J, Edgecombe B. Seroprevalence of Helicobacter pylori infection in adults in the Bahamas. West Indian Med J 2011;60:662–665.
2. Wang F, Meng W, Wang B, Qiao L. Helicobacter pylori-induced gastric inflammation and gastric cancer. Cancer Lett 2014;345:196–202.
3. Correa P, Pizuelo MB. The gastric precancerous cascade. J Dig Dis 2012;13:2–9.
4. Vannella L, Lahner E, Annibale B. Risk for gastric neoplasias in patients with chronic atrophic gastritis: a critical reappraisal. World J Gastroenterol 2012;18:1279–1285.
5. Warren JR. Gastric pathology associated with Helicobacter pylori. Gastroenterol Clin North Am 2000;29:705–751.
6. Lazár DC, Tában S, Cornianu M, Faur A, Goldiq A. New advances in targeted gastric cancer treatment. World J Gastroenterol 2016;22:6776–6799.
7. Gerhard M, Lehne N, Neumayer N, Börm T, Rad R et al. Clinical relevance of the Helicobacter pylori gene for blood-group antigen-binding adhesin. Proc Natl Acad Sci USA 1999;96:12778–12783.

8. Schmitt W, Haas R. Genetic analysis of the Helicobacter pylori vacuolating cytotoxin: structural similarities with the IgA protease type of exported protein. Mol Microbiol 1994;12:307–319.

9. Junaid M, Linn AK, Javadi MB, Ali-Gubare S, Ali N et al. Vacuolating cytotoxin A (VacA) - a multi-talented pore-forming toxin from Helicobacter pylori. Toxicon 2016;118:27–35.

10. Atherton JC, Cao P, Peek RM, Tummuru MK, Blaser MJ et al. Mosaicism in vacuolating cytotoxin alleles of Helicobacter pylori. Association of specific vacA types with cytotoxin production and peptic ulceration. J Biol Chem 1995;270:17771–17777.

11. Odendreit S, Püls J, Sedlmaier B, Gerland E, Fischer W et al. Translocation of Helicobacter pylori CagA into gastric epithelial cells by type IV secretion. Science 2000;287:1497–1500.

12. Stein M, Bagnoli F, Halenbeck R, Rappuoli R, Fantl WJ et al. c-Src/Lyn kinases activate Helicobacter pylori CagA through tyrosine phosphorylation of the EPIYA motifs. Mol Microbiol 2002;43:971–980.

13. Tammer I, Brandt S, Hartig R, König W, Backert S. Activation of Abl by Helicobacter pylori: a novel kinase for CagA and crucial mediator of host cell scattering. Gastroenterology 2007;132:1309–1319.

14. Backert S, Tegtmeier N, Sebald M. The versatility of Helicobacter pylori CagA effector protein functions: the master key hypothesis. Helicobacter 2010;15:163–176.

15. Higashi H, Tsutsuimi R, Fujita A, Yamazaki S, Asaka M et al. Biological activity of the Helicobacter pylori virulence factor CagA is determined by variation in the tyrosine phosphorylation sites. Proc Natl Acad Sci USA 2002;99:14428–14433.

16. Argent RH, Hale JL, El- Omar EM, Atherton JC. Differences in Helicobacter pylori CagA tyrosine phosphorylation motif patterns between western and east Asian strains, and influences on interleukin-8 secretion. J Med Microbiol 2008;57:1062–1067.

17. Ferreira RM, Machado JC, Leite M, Carneiro F, Figueiredo C. The number of Helicobacter pylori CagA EPIYA C tyrosine phosphorylation motifs influences the pattern of gastritis and the development of gastric carcinoma. Histopathology 2012;60:992–998.

18. Naito M, Yamazaki T, Tsutsuimi R, Higashi H, Onoe K et al. Influence of EPIYA-repeat polymorphism on the phosphorylation-dependent biological activity of Helicobacter pylori CagA. Gastroenterology 2006;130:1181–1190.

19. Alkout AM, Blackwell CC, Weir DM. Increased inflammatory responses of persons of blood group O to Helicobacter pylori. J Infect Dis 2000;181:1364–1369.

20. Rad R, Gerhard M, Lang R, Schöniger M, Rösch T et al. The Helicobacter pylori blood group antigen-binding adhesin facilitates bacterial colonization and augments a nonspecific immune response. J Immunol 2002;168:3033–3041.

21. Ilver D, Arnqvist A, Ogren J, Frick IM, Kersulyte D et al. Helicobacter pylori pylori adhesin binding fucosylated histo-blood group antigens revealed by retagging. Science 1998;279:373–377.

22. Ishijima N, Suzuki M, Ashida H, Ichikawa Y, Kanegae Y et al. BabA-mediated adherence is a potentiator of the Helicobacter pylori type IV secretion system activity. J Biol Chem 2011;286:25256–25264.

23. Stolte M, Meining A. The updated Sydney system: classification and grading of gastritis as the basis of diagnosis and treatment. Can J Gastroenterol 2001;15:591–598.

24. Sambrook J, MacCallum P, Russel D. Molecular Cloning: a Laboratory Manual, 3rd ed. NY, USA: Cold Spring Harbour Press; 2001.

25. Román-Román A, Giono-Cerezo S, Camorlinga-Ponce M, Martínez-Carrillo DN, Loaiza-Loeza S et al. vacA genotypes of Helicobacter pylori in the oral cavity and stomach of patients with chronic gastritis and gastric ulcer. Enferm Infecc Microbiol Clin 2013;31:130–135.

26. Slater E, Owen RJ, Williams M, Pounder RE. Conservation of the cag pathogenicity island of Helicobacter pylori: associations with vacuolating cytotoxin allele and IS605 diversity. Gastroenterology 1999;117:1308–1315.

27. Akopyants NS, Clifton SW, Kersulyte D, Crabtree JE, Youree BE et al. Analyses of the cag pathogenicity island of Helicobacter pylori. Mol Microbiol 1998;28:37–53.

28. Beltrán-Anaya FO, Poblete TM, Román-Román A, Reyes S, de Sampedro J et al. The EPIYA-ABCC motif pattern in CagA of Helicobacter pylori is associated with peptic ulcer and gastric cancer in Mexican population. BMC Gastroenterol 2014;14:223.

29. Paniagua GL, Monroy E, Rodríguez A, Arroniz S, Rodriguez C et al. Frequency of vacA, cagA and babA2 virulence markers in Helicobacter pylori strains isolated from Mexican patients with chronic gastritis. Ann Clin Microbiol Antimicrob 2009;8:14.

30. Chihu L, Ayala G, Mohar A, Hernández A, Herrera-Goepfert R et al. Antimicrobial resistance and characterization of Helicobacter pylori strains isolated from Mexican adults with clinical outcome. J Chemother 2005;17:270–276.

31. López-Vidal Y, Ponce-de-León S, Castillo-Rojas G, Barreto-Zúñiga R, Torre-Delgadillo A. High diversity of vacA and cagA Helicobacter pylori genotypes in patients with and without gastric cancer. PLoS One 2008;3:e3849.

32. Schultz M, Duarte I, Chianale J, Bravo R, Vergara MT et al. Frequency and histopathologic features of chronic gastritis in 300 patients without endoscopic lesions. Rev Med Chil 1996;124:545–552.

33. Suarez Ramos MdP, Martinez Baquero DL, Cabarcas Santoya ME, Ricaurte-Guerrero O. Gastropatía reactiva: frecuencia en biopsias endoscópicas evaluadas en la Universidad Nacional de Colombia. Rev Col Gastroenterol 2011;26:253–260.

34. Chen TS, Li AF, Chang FY. Gastric reddish streaks in the intact stomach: endoscopic feature of reactive gastropathy. Pathol Int 2010;60:298–304.

35. Zambon CF, Navaglia F, Basso D, Rugge M, Piebani M. Helicobacter pylori babA2, cagA, and s1 vacA genes work synergistically in causing intestinal metaplasia. J Clin Pathol 2003;56:287–291.

36. Ayala G, Flores-Luna L, Hernández-Amaro D, Mendoza- Hernández G, Chihu-Amparán L et al. Association of circulating VacA-neutralizing antibodies with gastric cancer and duodenal ulcer. Cancer Causes Control 2011;22:1425–1434.

37. Garza-Gonzalez E, Bosques-Padilla FJ, Tijerina-Menchaca R, Perez-Perez GI. Characterisation of Helicobacter pylori strains isolated from the north-eastern region of Mexico. Cln Microbiol Infect 2004;10:41–45.
gastric epithelial cells and its association with diversity in the cagA gene. *Infect Immun* 2007;75:3445–3454.

44. Sicinschi LA, Correa P, Peek RM, Camargo MC, Piazuelo MB et al. CagA C-terminal variations in *Helicobacter pylori* strains from Colombian patients with gastric precancerous lesions. *Clin Microbiol Infect* 2010;16:369–378.

45. Yamaoka Y, Kodama T, Gutierrez O, Kim JG, Kashima K et al. Relationship between *Helicobacter pylori* iceA, cagA, and vacA status and clinical outcome: studies in four different countries. *J Clin Microbiol* 1999;37:2274–2279.

46. Mattar R, dos Santos AF, Eisig JN, Rodrigues TN, Silva FM et al. No correlation of babA2 with vacA and cagA genotypes of *Helicobacter pylori* and grading of gastritis from peptic ulcer disease patients in Brazil. *Helicobacter* 2005;10:601–608.

47. Torres LE, Melián K, Moreno A, Alonso J, Sabatier CA et al. Prevalence of vacA, cagA and babA2 genes in Cuban *Helicobacter pylori* isolates. *World J Gastroenterol* 2009;15:204–210.

48. Rizzato C, Torres J, Plummer M, Muñoz N, Franceschi S et al. Variations in *Helicobacter pylori* cytotoxin-associated genes and their influence in progression to gastric cancer: implications for prevention. *PLoS One* 2012;7:e29605.

49. Zhang XS, Tegtmeier N, Traube L, Jindal S, Perez-Perez G et al. A specific A/T polymorphism in Western tyrosine phosphorylation B-motifs regulates *Helicobacter pylori* CagA epithelial cell interactions. *PLoS Pathog* 2015;11:e1004621.

50. Chang YH, Wang L, Lee MS, Cheng CW, Wu CY et al. Genotypic characterization of *Helicobacter pylori* cagA and vacA from biopsy specimens of patients with gastroduodenal diseases. *Mt Sinai J Med* 2006;73:622–626.

51. Yamaoka Y, Kodama T, Kita M, Imanishi J, Kashima K et al. Relationship of vacA genotypes of *Helicobacter pylori* to cagA status, cytotoxin production, and clinical outcome. *Helicobacter* 1998;3:241–253.

52. Argent RH, Zhang Y, Atherton JC. Simple method for determination of the number of *Helicobacter pylori* CagA variable-region EPIYA tyrosine phosphorylation motifs by PCR. *J Clin Microbiol* 2005;43:791–795.

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