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Frequency of *vacA*, *cagA* and *babA2* virulence markers in *Helicobacter pylori* strains isolated from Mexican patients with chronic gastritis

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Abstract

Background: *Helicobacter pylori* has been strongly associated with chronic gastritis, peptic and duodenal ulcers, and it is a risk factor for gastric cancer. Three major virulence factors of *H. pylori* have been described: the vacuolating toxin (VacA), the cytotoxin-associated gene product (CagA) and the adhesion protein BabA2. Since considerable geographic diversity in the prevalence of *H. pylori* virulence factors has been reported, the aim of this work was to establish the *H. pylori* and *vacA*, *cagA* and *babA2* gene status in 238 adult patients, from a marginal urban area of Mexico, with chronic gastritis.

Methods: *H. pylori* was identified in cultures of gastric biopsies by nested PCR. *vacA* and *cagA* genes were detected by multiplex PCR, whereas babA2 gene was identified by conventional PCR.

Results: *H. pylori*-positive biopsies were 143 (60.1%). All *H. pylori* strains were *vacA*$^+$; 39.2% were *cagA*$^+$; 13.3% were *cagA*$^+$ *babA2*$^+$ and 8.4% were *babA2*$^+$. Mexican strains examined possessed the *vacA s1, m1* (43.4%), *s1, m2* (24.5%), *s2, m1* (20.3%) and *s2, m2* (11.9%) genotypes.

Conclusion: These results show that the Mexican patients suffering chronic gastritis we have studied had a high incidence of infection by *H. pylori*. Forty four percent (63/143) of the *H. pylori* strains analyzed in this work may be considered as highly virulent since they possessed two or three of the virulence markers analyzed: *vacA s1 cagA babA2* (9.8%, 14/143), *vacA s1 babA2* (4.9%, 7/143), and *vacA s1 cagA* (29.4%, 42/143). However, a statistically significant correlation was not observed between *vacAs1*, *cagA* and *babA2* virulence markers (χ^2 test; $P > 0.05$).

Background

Helicobacter pylori is a spiral-shaped Gram-negative bacterium that has been strongly associated with chronic gastritis and peptic ulcer disease [1,2], and it is a risk factor for gastric cancer [3-5]

Three major virulence factors of *H. pylori* have been described: the cytotoxin-associated gene product (CagA), the vacuolating toxin (VacA) and the adhesion protein BabA2. The cytotoxin-associated gene A (CagA) is a protein with a molecular mass of approximately 125–140 kDa, encoded by the *cagA* gene, [6,7], that is translocated into gastric epithelial cells by a type IV secretion system, encoded by the *cag* pathogenicity island (*cag* PAI) [8]. Inside epithelial cells CagA is phosphorylated on tyrosine residues by host cell Src kinases and stimulates cell-signaling pathways [9], which in turn causes elongation of the cell [10] and activation of proto-oncogenes [11].

The vacuolating cytotoxin gene *vacA* is polymorphic, varying in the signal and middle regions. The main signal region alleles are *s1* and *s2*, whereas the middle region alleles are *m1* and *m2* [12,13]. VacA is a toxin that binds to several epithelial receptors [14-16] and forms hexameric pores [17], which later are endocytosed and converted in large vacuoles [18].

The BabA adhesin of *H. pylori* is an outer membrane protein that binds to the fucosylated histo-blood group antigens on the surface of gastric epithelial cells [19,20]. It has been reported that *H. pylori* strains possessing *babA2* gene, which encodes active BabA adhesin, are associated with

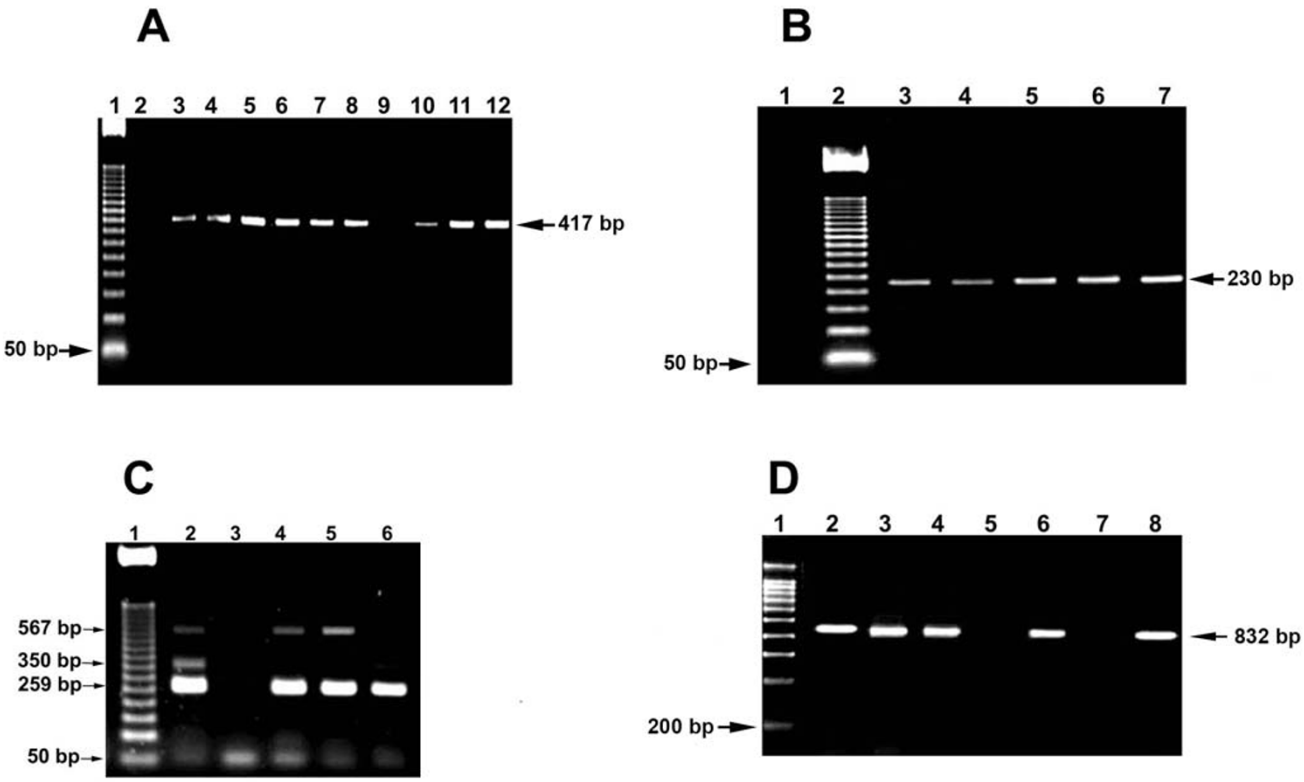

Figure 1

Identification of *H. pylori* isolated from gastric biopsy samples and genotyping of its main virulence genes by PCR. Images shown are from representative gel electrophoresis. A: Lane 1, MWM 50 bp-ladder; Lanes 2 and 9 negative control without DNA; Lanes 3–8, 10 and 11, *H. pylori* isolated from gastric biopsy samples (417-bp amplicon); Lane 12, reference strain *H. pylori* ATCC 43629. B: Lane 1, negative control without DNA, Lane 2, MWM 50 bp-ladder; Lanes 3–6, *H. pylori* isolated from gastric biopsy samples (230-bp amplicon); Lane 7, *H. pylori* ATCC 43629. C: Lane 1, MWM 50-bp-ladder; Lane 2, *H. pylori* isolated from gastric biopsy sample (*vacA s1, m1 cagA*); Lane 3, negative control without DNA; Lanes 4–5, *H. pylori* isolated from gastric biopsy samples (*vacA s1, m1*); Lane 6, ATCC 43629. D: Lane 1, MWM 200 bp-ladder; Lane 2, ATCC 43629; Lanes 3,4,6 and 8, *babA2*-positive *H. pylori* isolated from gastric biopsy samples; Lane 5, control without DNA; Lane 7, *babA2*-negative *H. pylory* isolated from gastric biopsy sample.

Table 1: Frequency of *vacA H. pylori* genotypes in Mexican patients with chronic gastritis

Genotype	Number (%)
s1m1	62 (43.4)
s1m2	35 (24.5)
s2m1	29 (20.3)
s2m2	17 (11.9)
	143 (100)

increased gastric inflammation [21] and increased risk for duodenal ulcer and adenocarcinoma [22].

H. pylori virulence factors frequency varies widely. For instance, *vacAs1* prevalence fluctuates from 48% [23] to 100% [24] whereas *cagA* prevalence fluctuates from 66.9% [23] to 83.6% [25] and 100% [26]. *babA2* reported frequencies vary from 46% [27] to 82.3% [28] in South-American countries. Since considerable geographic diversity in the prevalence of *H. pylori* virulence factors has been reported, the aim of this work was to establish the *H. pylori* and *vacA*, *cagA* and *babA2* gene status in 238 adult patients, from a marginal urban area of Mexico, with chronic gastritis.

Materials and methods

Subjects and clinical samples

Two hundred and thirty eight patients, endoscopic diagnosed with chronic gastritis (154 women and 84 men) with an average age of 52.24 years (range 16 to 83), who had undergone endoscopy in Hospital General Regional 72 of the Instituto Mexicano del Seguro Social at Tlalnepantla, Estado de Mexico, Mexico, were included in this study. Written informed consent for participation was obtained from every patient before the study. The ethics committee at Hospital General Regional 72 approved the study protocol in advance. Antral biopsy specimens were evaluated for the presence of *H. pylori* by culture. The genotype profiles of *H. pylori* isolates were determined by PCR.

Table 2: Frequency of *cagA* and *babA2* genes in *H. pylori* isolates

Genotype			Number (%)
vacA	*cagA*	*babA2*	
+	+	+	19 (13.3)
+	-	+	12 (8.4)
+	+	-	56 (39.2)
+	-	-	56 (39.2)
			143 (100)

H. pylori *culture*

For bacterial culture, biopsy specimens were macerated and homogenized in Brucella Broth and a 100 μL aliquot was inoculated on Casman Agar (Difco) containing 5% horse blood and *H. pylori* selective supplement (Oxoid-SR 147E). Agar plates were incubated in 6% CO_2, for up to four days. Colonies were identified as *H. pylori* according to standard criteria including negative Gram staining, typical cell morphology, and positive reactions to catalase, oxidase, and urease.

Identification of H. pylori *by nested PCR*

H. pylori DNA was extracted from colonies collected in microcentrifuge tubes containing 125 μL of sterile phosphate-buffered saline. Suspensions were vortexed vigorously for 2 min; the tubes were boiled in a water bath for 15 min, cooled in ice, and centrifuged at 13000 × g for 1 min. DNA in supernatant was stored at -20°C until used as template in PCR.

H. pylori was detected by nested PCR. First PCR run was done as described by Li et al., [29] with primers EHC-U (5'-CCCTCACGCCATCAGTCCCAAAAA-3') and EHC-L (5'-AAGAAGTCAAAAACGCCCCAAAAC-3'). Amplification was performed in 25 μL reaction volume containing 1 μL (25 pmol) of each primer (EHC-U and EHC-L, Sigma-Genosys), 2.5 μL 10× Buffer Solution, 17.5 μL nuclease-free water, 3 μL template DNA, 1.5 mmol $MgCl_2$, 0.5 U AmpliTaq polimerase and 100 mmol dNTPs (PuReTaqTM Ready-To-GoTM PCR beads). Products were amplified under the following conditions: 5 min at 95°C for initial denaturation followed by 40 cycles of 45 s at 94°C, 45 s at 59°C, and 30 s at 72°C with a final round of 10 min at 72°C in a Corbett Research CGI-96 Thermocycler. A 417 bp product was obtained by this procedure. Second PCR run was done as described by Song et al., [30] with primers ET-5U (5'-GCCAAATCATAAGTCCGCAGAA-3') and ET-5L (5'-TGAGACTTTCCTAGAAGCGGTGTT-3') complementary to an internal fragment of the amplicon obtained with EHC-U and EHC-L primers. Amplification conditions were identical to those of the first run, except that 0.2 μL of the first PCR run product as template, and 25 cycles, were used. A 230 bp amplicon was obtained.

In each experiment, both positive and negative controls, with DNA from H. pylori ATCC 43629 and without template DNA, were included.

Detection of cagA, vacA, *and* babA2 *by PCR*

In order to detect *cagA* and *vacA* alleles, primers and multiplex PCR amplification conditions described by Chattopadhyay, et al., [31] and Atherton et al., [13,32] were used. These PCR protocols detect *cagA* (350 bp amplicon) and distinguish *vacA s1* (259 bp amplicon) from *vacA s2* (286 bp amplicon), and *vacA m1* (567 bp amplicon) from

Table 3: Frequency of *cagA* an *babA2* genes in *vacAs1*-positive *H. pylori* isolates

Genotype			Number (%)
vacAs1	*cagA*	*babA2*	
+	+	+	14 (14.4)
+	-	+	7 (7.2)
+	+	-	42 (43.3)
+	-	-	34 (35.0)
			97 (100)

vacA m2 (642 bp amplicon). PCR detection of *babA2* was done as described by Gerhard et al., [22]. PCR products were analyzed by agarose gel electrophoresis at 120 V, 94 mA for 120 min. Gels were stained with ethidium bromide and photographed under UV illumination with Gel Logic 100 system (Kodak).

Results and discussion

It is known that more than 50% of the world's human population is colonized by *H. pylori* [33,34]. We report here that *H. pylori* was cultured from 60.1% biopsy samples (143/238) and identified by nested PCR, which rendered the expected 417 bp and 230 bp amplicons (Fig 1A, B) as reported by Li et al., [29] and Song et al., [30]. This result is in agreement with previously reported *H. pylori* prevalence in Mexican people. A community-based national seroprevalence survey of *H. pylori* infection in Mexico showed an overall prevalence of 66%. Twenty-percent of one-year-old children had antibodies against *H. pylori*, with an increased seropositivity of up to 50% in children who were 10 years of age [35]. Variations in prevalence have been reported among particular regions with a prevalence of 86.1% in southeastern Mexico [36] and 47.1% in children from northwestern Mexico [37].

All *H. pylori* strains were positive for the *vacA* gene (Table 1), as evidenced by PCR product sizes, which enabled to differentiate *s* and *m* alleles (Fig. 1C). *s1 m1* was the most frequent *vacA* allelic combination in the *H. pylori* strains examined, followed by *s1 m2*, *s2 m1* and *s2 m2* (Table 1). These results suggest that two thirds of these strains are virulent, as it has been reported that *H. pylori* isolates with *vacAs1m1* and *vacAs1m2* allelic combinations exhibit high and low vacuolating activity, respectively, whereas those with *vacAs2* fails to induce cell vacuolation in vitro [13].

Amplicons of the *cagA* and *babA2* genes were detected in agarose gels as 350 bp and 832 bp bands, respectively, (Fig. 1C and 1D). Fifty two percent of the *H. pylori* isolates were *cagA*-positive, prevalence less than that reported in other studies from South-American [28,38,39] and Asian countries [40]. In Mexico, prevalence of *H. pylori* infection with CagA-positive strains varies from 47.6% to 63.4% [41]. *cagA*-positive *H. pylori* strains have been associated with the severe mucosa inflammation that underlies peptic ulcer, atrophic gastritis and gastric carcinoma [42-44].

Although *vacAs1 cagA+ H. pylori* strains had been considered as virulent, in a study of *H. pylori* isolates from Mexican patients it was reported that *vacAs1b* and *cagA+* strains were found at similar frequencies in adults with and without peptic ulcers [45].

H. pylori BabA adhesin, encoded by the *babA2* gene, participates in adhesion of *H. pylori* to Le^b antigens on human gastric epithelial cells [19]. The *babA2* gene was found in only 21.7% of the *H. pylori* isolates (Table 2). This frequency is considerably lesser than *babA2* reported frequencies, which vary from 46% [27] to 82.3% [28] in South-American countries. It is important to note that PCR detection of *babA2* in *H. pylori* do not always correlates with its adhesive properties and, conversely, failure to detect *babA2* by PCR does not mean that the strain is not adherent, as there is substantial allelic variation in *babA2* gene [46-48].

Forty four percent of the *H. pylori* strains analyzed in this work (63/143) possessed two or three of the virulence markers analyzed (Table 3): *vacA s1 cagA babA2* (9.8%, 14/143), *vacA s1 babA2* (4.9%, 7/143), and *vacA s1 cagA* (29.4%, 42/143).

Conclusion

Results presented here show that the Mexican patients suffering chronic gastritis we have studied had a high incidence of infection by *H. pylori*, and suggest that 44% of the *H. pylori* strains examined may be considered virulent, since they possessed two or three of the virulence markers analyzed. However, a statistically significant correlation was not observed between *vacAs1*, *cagA* and *babA2* virulence markers (χ^2 test; $P > 0.05$).

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

GP, EM and EN carried out the molecular studies; RR, JC and AC, obtained the gastric biopsy samples; SA and CR carried out the microbiological procedures; SV conceived of the study, and participated in its design and coordination, and drafted the manuscript. All authors read and approved the final manuscript.

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