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ORIGINAL ARTICLE

Frequency of virulence genes in mixed infections with *Helicobacter pylori* strains from a Mexican population☆

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KEYWORDS
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Virulence factors;
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Random Amplified Polymorphic DNA

Abstract
Background: Helicobacter pylori (*H. pylori*) is associated with gastroduodenal diseases. Virulence of clinical isolates is related to clinical outcome. Moreover, with microdiversity studies in clinical isolates from a single patient, but from a different origin (antrum or corpus), it is possible to demonstrate that there are simultaneous mixed infections.
Aims: To genotype *H. pylori* strains with multiplex PCR, according to their clinical virulence, and in this manner know the frequency of each genotype and relate it to clinical outcome in order to prevent the development of severe diseases.
Methods: A total of 210 patients with gastric alterations were studied. Virulence classification of *H. pylori* strains was carried out with multiplex PCR and 127 strains were identified as *H. pylori* by PCR (*glmM* and *cagE*). Genotype and clinical outcome were evaluated with the Fisher's exact test. In addition, RAPD-PCR was performed as a fingerprinting method to analyze mixed infections.

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Results: The *cagA*, *vacAs*$_1$, and *vacAm*$_1$ genes were detected in all the clinical isolates. Strains were classified as: type I, 40.15% (51/127); type II, 22.04% (28/127); and type III, 28.4% (36/127), but two new different genotypes were also detected: (1) *babA*$_2$+, *cagA*+, *vacAs*$_1$+, 6.29% (8/127) and (2) *babA*$_2$+, *cagA*-, *vacAs*$_2$/*m*$_2$+, 3.14% (4/127). The *cagE* gene was detected in type I strains.
Conclusions: The Fisher's exact test did not support a significant association between clinical outcome and genotype. The main circulating genotypes in the Mexican population studied were: *cagA*+, *vacAs*$_1$, and *vacAm*$_1$. Multiplex PCR can be used as a screening test for *H. pylori* strains. Furthermore, the *cagE* gene is a good marker for identifying cag-PAI positive strains.

PALABRAS CLAVE
Helicobacter pylori;
Factores de virulencia;
Genotipificación;
Infección mixta;
ADN polimórfico amplificado al azar

Frecuencia de genes de virulencia en infecciones mixtas con cepas de *Helicobacter pylori* de una población mexicana

Resumen
Antecedentes: La infección por *Helicobacter pylori* se asocia con gastritis. La variabilidad genética de las cepas tiene importancia clínica. Los estudios de microdiversidad en cepas aisladas de diferente sitio anatómico en un mismo paciente revelan la posibilidad de recuperar 2 o más cepas diferentes.
Objetivos: Genotipificar por PCR multiplex según la virulencia de aislados clínicos de *H. pylori* para determinar la frecuencia de cada genotipo y relacionarlo con la evolución clínica con la finalidad de prevenir el desarrollo de enfermedades graves.
Métodos: Se estudió a 210 pacientes con alteraciones gastroduodenales; 127 cepas fueron identificadas por PCR *(glmM* y *cagE)* y después clasificadas según su virulencia por PCR multiplex. Se hizo la prueba de Fisher para evaluar la relación entre genotipo y resultado clínico. La técnica de RAPD-PCR se empleó como método de huella genética y para analizar la presencia de infecciones mixtas.
Resultados: cagA, *vacAs*$_1$ y *vacAm*$_1$ estuvieron presentes en todos los aislados clínicos. Las cepas se clasificaron como: tipo I, 40.15%(51/127); tipo II, 22.04% (28/127), y triples positivas, 28.4% (36/127); se detectaron 2 genotipos nuevos: *1) babA*$_2^+$, *cagA*+, *vacAs*$_1^+$, 6.29% (8/27), y *2) babA*$_2^+$, *cagA*$^-$, *vacAs*$_2$/*m*$_2^+$, 3.14% (4/127). *cagE* se detectó en las cepas tipo II.
Conclusiones: La prueba de Fisher no mostró una asociación significativa entre el resultado clínico y el genotipo en la población estudiada. Los genotipos circulantes en la población mexicana fueron *cagA*+, *vacAs*$_1$, *vacAm*$_1$. La PCR multiplex puede usarse para genotipificar rápidamente las cepas de *H. pylori*. *cagE* es un buen marcador para identificar cepas *cag*-PAI+.

Introduction

Helicobacter pylori (*H. pylori*) is a Gram-negative, spiral-shaped, microaerophilic bacterium associated with gastritis and peptic ulcer disease (PUD) and is considered a risk factor for gastric cancer.[1–2] Half of the world population is infected with this bacterium, but only 10% of infected people will develop clinical outcomes. Infections with *H. pylori* may induce chronic gastritis, PUD, and gastric adenocarcinoma.[3] There is evidence showing that the genetic variability of *H. pylori* strains has clinical importance. Three major virulence factors are used as epidemiological markers and to determine clinical outcome: the genes *vacA* (vacuolating toxin), *cagA* (cytotoxin-associated gene), and *babA* (BabA adhesin).[4]

The *vacA* gene, encoding the vacuolating toxin (VacA), is present in almost all strains. Significant polymorphism within *vacA* can be found in the middle region (*m)*, with 2 alleles: m_1 or m_2 as well as in the signal sequence region (*s*) with 3: s_{1a}, s_{1b}, or s_2. The possible genotypes are: $s_{1b}m_1$, $s_{1a}m_1$, $s_{1a}m_2$, $s_{1b}m_2$, s_2m_1, and s_2m_2, although the s_2m_1 genotype is reported to be rare.[5] The $s_{1a}m_1$ genotype, rather than m_2, is associated with PUD.[3] A third polymorphic determinant of vacuolating activity has been described and is called the intermediate (*i*) region. There are 2 *i*-region subtypes: i_1 and i_2 and variation among populations has been observed. Moreover, studies have shown a strong relation between the i_1 allele and the production of CagA and the presence of the s_1 type allele in various *H. pylori* strains isolated in individuals from several countries. This association could indicate that the intermediate region plays a role in more severe outcomes of chronic *H. pylori* infection.[6] CagA is associated with an increased risk for gastric cancer. It is a protein that is injected into the interior of the cell through a type