

# HELICOBACTER PYLORI INFECTION IN PATIENTS WITH DIFFERENT GASTROINTESTINAL DISEASES FROM NORTHERN BRAZIL

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Received 21/4/2015  
Accepted 18/6/2015

**ABSTRACT - Background** - The mechanisms whereby *Helicobacter pylori* produces different pathological manifestations in the stomach and duodenum are not fully understood. Considering the geographic diversity in the prevalence of virulence factors of this microorganism and their association with the development of different diseases, the search for pathogenicity markers such as CagA and VacA alleles by molecular techniques has intensified. **Objectives** - To investigate the presence of *H. pylori* infection and the frequency of different genotypes of this bacterium in patients with gastrointestinal diseases from Northern Brazil, and to establish their association with the histopathological findings. **Methods** - In a prospective study, samples were collected from 554 patients with different gastrointestinal diseases (gastritis, duodenal ulcer, gastric ulcer, and gastric cancer) seen at a referral hospital attending the entire State of Pará, located in the metropolitan region of Belém. Data such as gender and age obtained with an epidemiological questionnaire were analyzed. The presence of *H. pylori* and the bacterial genotype were investigated by PCR. Gastric biopsies were assessed histologically. **Results** - The prevalence of *H. pylori* infection was 91%. Infection was more frequent among patients with gastric ulcer and gastric cancer. In these groups, there was a predominance of men and older patients when compared to the other two groups studied. The predominant bacterial genotype was s1m1cagA+, which was more frequent among patients with gastric ulcer, duodenal ulcer and gastric cancer. A significant association was observed between s1m1cagA+ strains and a higher degree of inflammation, neutrophil activity and development of intestinal metaplasia. **Conclusion** - The present study demonstrates a high incidence of *H. pylori* infection in the patients analyzed, especially among those with gastric ulcer and gastric cancer. Virulent s1m1cagA+ strains predominated and were associated with more severe lesions.

**HEADINGS** - *Helicobacter pylori*. *Helicobacter* infections. Gastrointestinal diseases. Genotype.

## INTRODUCTION

Infection with *Helicobacter pylori* induces persistent inflammation in the gastric mucosa, causing gastritis in almost all infected individuals. Although most hosts remain asymptomatic, others develop peptic or duodenal ulcers, gastric adenocarcinoma and lymphomas, especially mucosa-associated lymphoid tissue (MALT) lymphoma<sup>(26)</sup>. The mechanism whereby the bacterium produces different pathological manifestations in the stomach and duodenum are not fully understood. In this respect, bacterial virulence factors, environmental factors and host genetic factors probably contribute to the differences in clinical evolution<sup>(5)</sup>.

Virulence factors play an important role in the pathogenesis of *H. pylori* infection, since virulent strains are more aggressive and increase the risk of

developing severe clinical manifestations<sup>(6)</sup>. The most important *H. pylori* virulence factor is the cytotoxin-associated gene pathogenicity island (cag PAI). Cytotoxin-associated gene A (CagA) is a protein with a molecular mass of approximately 125–140 kDa, which is encoded by the cagA gene. The protein is translocated into gastric epithelial cells by a type IV secretion system, encoded by the cag pathogenicity island (cag PAI)<sup>(23)</sup>. Inside epithelial cells, CagA is phosphorylated on tyrosine residues by host cell Src kinases and stimulates cell signaling pathways<sup>(29)</sup> which, in turn, cause elongation of the cell<sup>(30)</sup> and activation of proto-oncogenes<sup>(19)</sup>.

VacA, or vacuolating cytotoxin, is another virulence factor that plays a key role in the pathogenesis of *H. pylori*. Three cellular activities of this protein have been confirmed: cell vacuolation, apoptosis, and CD4+ T-lymphocyte activation and proliferation<sup>(27)</sup>.

Declared conflict of interest of all authors: none  
Disclosure of funding: no funding received

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Specific variants of the *vacA* allele exhibit different levels of toxin activity and are associated with different risks of gastrointestinal diseases. There are two types of signal regions (s1 and s2) and mid-regions (m1 and m2) that possess different vacuolating activities. In general, genotypes *vacA* s1 and m1 produce large amounts of toxin and induce greater vacuolating activity in the gastric epithelium, while genotypes s2 and m2 produce little or no toxin. *VacA* s2-m2 strains are rarely associated with the development of peptic ulcer and gastric cancer<sup>(32)</sup>.

*Helicobacter pylori* strains have been divided into two groups: type I (*cag+*/s1m1 *VacA*) that causes ulceration and cancer, and type II (*cag-*/s2m2 *VacA*) that induces less inflammation and tissue damage<sup>(26)</sup>.

Since considerable geographic diversity in the prevalence of *H. pylori* virulence factors has been reported, the objective of the present study was to investigate the presence of *H. pylori* infection and the frequency of association of different bacterial genotypes with gastrointestinal diseases in 554 patients seen at a referral hospital in the Metropolitan Region of Belém, Pará, Brazil, as well as their association with different histopathological findings. The hospital receives patients from across the State of Pará.

## METHODS

### Patients

Gastric biopsies were collected from 554 patients with different gastrointestinal disorders seen at the endoscopy sector of the Ofir Loiola Hospital from 2013 to 2014. This Hospital is a reference in cancer treatment and receives patients from throughout the region suspected of having this disease for diagnostic investigation.

A specific questionnaire elaborated and applied by the authors and completed by the patients was used to obtain data such as gender, age, and symptoms. The patients agreed to participate in the study by signing a free informed consent form.

During endoscopy, four biopsy fragments were obtained from the stomach of each patient. Biopsies of the lesion and perilesional mucosal area were obtained from each patient for histological analysis and two antrum specimens were also analyzed using molecular methods. None of the patients had received antimicrobial drugs, H2-receptor antagonists, acid pump inhibitors, nonsteroidal anti-inflammatory drugs, or any medication for at least 60 days prior to endoscopy.

All patients included were of low socioeconomic level and had similar cultural habits. All patients were born in Pará State and had the same ethnic background, approximately 50% Portuguese, 40% Amerindian and 10% African<sup>(28)</sup>. The study was approved by the Ethics Committee of the Center of Tropical Medicine, Federal University of Pará.

### Extraction of DNA and PCR

Gastric fragments were collected from the antrum of each patient for histopathological analysis and extraction of bacterial DNA<sup>(17)</sup>. Total DNA was extracted from frozen

gastric biopsy specimens using the following procedure: 10 mL proteinase K and 300 mL lysis buffer (200 mM Tris-HCl, 25 mM EDTA, 300 mM NaCl, 1.2% sodium dodecyl sulfate) were added to the biopsy specimens and the mixture was incubated at 55°C for 12 h. The lysate was extracted with an equal volume of phenol-chloroform, precipitated with isopropanol, and washed with 70% ethanol. The pellet was dried and suspended in 200 mL sterile distilled water. The extracted DNA was stored at -20°C.

A set of primers (p1 and p2)<sup>(13)</sup> that amplify a gene fragment of 298 bp found in all *H. pylori* strains was used to detect bacterial DNA. Only positive samples were used for amplification of the *cagA* gene.

The protocol and primers described by Tummuru et al.<sup>(34)</sup> were used for amplification of the *cagA* gene. Negative and positive controls were included in all reactions. The PCR products were separated by electrophoresis on 2% agarose gel stained with ethidium bromide and visualized under a UV transilluminator.

### Histopathological analysis

Hematoxylin-eosin staining was used for histopathological analysis of the gastric biopsies. The histopathological parameters were scored from 0-3 according to criteria of the Sydney System for analysis of polymorphonuclear and mononuclear cell infiltration<sup>(20)</sup>.

### Statistical analysis

The chi-square test and G test were used to compare frequencies between groups. Risk factors were estimated by univariate analysis using odds ratios. Statistical significance was accepted at a level of 95%. The BioEstat 5.0 program was used for data analysis<sup>(4)</sup>.

## RESULTS

A total of 554 patients with different gastrointestinal diseases were analyzed; of these, 49.3% (273/554) had gastritis, 9.9% (55/554) had gastric ulcers, 13.4% (74/554) had duodenal ulcers, and 27.4% (152/554) had gastric adenocarcinoma. Of the 273 patients with gastritis, 73% (200/273) were superficial type, 22% (61/273) erosive and 5% (12/273) atrophic.

### Epidemiological and histopathological data

Table 1 shows the comparison of the frequency of gender, age, *H. pylori* infection and histopathological data between groups. Among the patients studied, 58.1% (322/554) were males and 41.9% (232/554) were females. There was a predominance of male patients among those with gastric ulcer ( $\chi^2=8.81, P<0.01$ ) and gastric cancer ( $\chi^2=7.38, P<0.01$ ) when compared to patients with gastritis and duodenal ulcer.

Patient age ranged from 18 to 91 years, with a mean age of 43.8 years in patients with gastritis, 43 years in patients with duodenal ulcer, 53.8 years in patients with gastric ulcer, and 51.5 years in the group with gastric cancer. Patients with gastric ulcer and cancer were older than those of the other two groups studied ( $\chi^2=4.5, 6 P<0.01$ ).

TABLE 1. Epidemiological and histopathological data of patients with different gastrointestinal diseases

Variables	GS n=273 (%)	UD n=74 (%)	UG n=55 (%)	CA n=152(%)
Sex				
Male	141 (51.6)	40 (54)	41 (74.5)	100 (65.8)
Female	132 (48.4)	34 (46)	14 (25.5)	52 (34.2)
<i>P</i> *	ns	ns	<0.01	<0.01
Age				
50 years	78 (28.6)	19 (25.7)	33 (60)	73 (48)
<50 years	195 (71.4)	55 (74.3)	22 (40)	79 (52)
<i>P</i> *	ns	ns	<0.01	<0.01
<i>H. pylori</i>				
Positive	244 (89.4)	66 (89.2)	51 (92.7)	145 (95.4)
Negative	26 (10.6)	8 (10.8)	4 (7.3)	7 (4.6)
<i>P</i> *	-	ns	<0.01	<0.01
Histopathological data				
DI				
1	130 (47.2)	18 (23.9)	16 (29.1)	23 (15.13)
2 e 3	143(51.8)	56 (76.1)	39 (70.9)	129 (84.87)
<i>P</i> *	-	<0.01	<0.01	<0.01
NA				
1	118 (43.3)	17 (22.9)	13 (23.6)	22 (14.5)
2 e 3	155 (56.7)	57 (77.1)	42 (76.4)	130 (85.5)
<i>P</i> *	-	<0.01	<0.01	<0.01

G test ns: not significant; DU: duodenal ulcer; GU: gastric ulcer; GC: gastric cancer; DI: degree of inflammation; NA: neutrophil activity. 1=mild, 2=moderate, 3=intense.

In the histological analysis, 34% (187/554) of the biopsies showed mild degree of inflammation, 37% (207/554) moderate, and 29% (160/554) intense. Neutrophil activity in 30% of biopsies (170/554) were mild, 39% (214/554) were moderate and 31% (170/554) intense. Comparing the histopathological data between different diseases studied, it was observed that patients with duodenal ulcer, gastric ulcer and gastric adenocarcinoma exhibited a higher degree of inflammation and neutrophil activity than patients with gastritis.

Investigation of *H. pylori* infection by a molecular biology method (PCR) detected bacterial DNA in gastric biopsies of 91% (506/554) of the cases analyzed. *Helicobacter pylori* was detected in 89.4% (244/273) of patients with gastritis, 89.2% (66/74) of patients with duodenal ulcer, 92.7% (51/55) of patients with gastric ulcer, and 95.4% (145/152) of patients with gastric cancer.

### Genotyping of *H. pylori vacA* and *cagA* genes

Characterization of the *vacA* alleles of the bacterial strains showed that all isolates carried the *vacA* gene. Among the patients analyzed, 97.6% (494/506) were colonized with only one strain, which exhibited only one *vacA* genotype (s1/m1 or s1/m2 or s2m2), and 2.4% (12/506) were infected with more than one strain, with the detection of more than one *vacA* genotype (s1/m1 and s2/m2).

Analysis of the *vacA* gene showed that 88% (446/506) of the patients carried allele s1 of the signal region of the gene. There was a predominance of allele m1 of the mid-region in 87% (440/506) of the patients. In patients with mono-infection, 86.6% (428/494) of the bacterial strains detected carried

the s1/m1 genotype, 1.2% (6/494) the s1/m2 genotype, and 12.2% (60/494) the s2/m2 genotype.

The *cagA* gene was detected in 85.6% (433/ 506) of the patients infected with *H. pylori*. With respect to gastrointestinal diseases, the presence of the *cagA* gene was observed in 80.3% (196/244) of patients with gastritis, in 98.5% (65/66) of patients with duodenal ulcer, in 88.2% (45/51) of patients with gastric ulcer, and in 88.3% (128/145) of patients with gastric cancer.

Comparison of the different *vacA* alleles and presence or absence of *cagA* showed a higher prevalence of virulent s1m1cagA+ strains in patients with mono-infection. A predominance of the s1m1/s2m2cagA+ genotype was observed in patients infected with multiple strains. Another genotype found in mixed infections was s1m1/s2m2cagA- (Table 2).

TABLE 2. Frequency of different bacterial genotypes detected in all patients studied

Genotype	Frequency	%
Mono-infection		
s1m1cagA+	422	83.4
s1m1cagA-	6	1.2
s1m2cagA-	6	1.2
s2m2cagA-	60	11.8
Mixed infection		
s1m1/s2m2 cagA+	11	2.2
s1m1/s2m2 cagA-	1	0.2
Total	506	100.0

### Frequency of different genotypes in patients with gastrointestinal diseases

Analysis of the correlation between the endoscopic diagnosis of gastrointestinal disease and *H. pylori* strains showed that virulent s1m1cagA+ strains were more frequent in patients with gastric ulcer (G=34.18; P=0.01), duodenal ulcer (G=62.04; P=0.01), and gastric cancer (G=2.29; P=0.12) compared to patients with gastritis. The comparison of strains between the group with gastric ulcer and the groups with duodenal ulcer (G=62.04; P=0.01) and gastric cancer (G=0.016; P=0.89) was not significant. Table 3 shows the frequency of the different genotypes in patients with gastrointestinal diseases.

TABLE 3. Prevalence of cytotoxin-associated gene (cagA) and allele combinations of the vacA gene in patients with different gastrointestinal diseases

Genotype	Gastritis % n=244	GU % n=51	Cancer % n=145	DU % n=66
Monoinfection				
s1-m1-cagA+	188 (77)	44 (86.3)	126 (86.9)	64 (97)
NV	48 (19.7)	6 (11.8)	16 (11)	2 (3)
Mixed infection				
s1 m1\s2 m2 cagA+	8 (3.3)	1 (1.9)	2 (1.4)	-
s1 m1\s1 m2 cagA-	-	-	1(0.7)	-

GU: gastric ulcer; DU: duodenal ulcer; NV: non-virulent strain (s1-m1-cagA-, s1-m2-cagA-, s2-m2-cagA-).

### Association between *H. pylori* genotypes and histopathological findings

Only patients with monoinfection (n=494) were studied since the number of patients with mixed infection was not statistically significant for this analysis. Patients infected with s1m1cagA+ strains exhibited a higher degree of inflammation and neutrophil activity than patients infected with other strains (Table 4).

TABLE 4. Association of the different vacA alleles and presence of the cagA gene with histopathological data (degree of inflammation and neutrophil activity) in monoinfected patients

vacA Alleles	cagA	DI		OR/P-value (95% CI)	NA		OR/P-value (95% CI)
		1	2 and 3		1	2 and 3	
s1m1	(+)	96	326	-	86	336	
s1m1	(-)	1	5	8.24/0.01	3	3	11.72/0.01
s1m2	(-)	6	-	(4.72-14.39)	6	-	(6.53-21.01)
s1m2	(-)	44	16		45	15	

OR: odds ratio; 95% CI: 95% confidence interval; DI: degree of inflammation; NA: neutrophil activity. 1=mild, 2=moderate, 3=intense.

Metaplasia was observed in 24.5% (121/494) of patients with monoinfection. The bacterial genotype s1m1cagA+ was identified in the gastric mucosa of most of these patients (Table 5).

TABLE 5. Association of the different vacA alleles and presence of the cagA gene with intestinal metaplasia

vacA Alleles	cagA	Metaplasia		OR/P-value (95% CI)
		Present	Absent	
s1m1	(+)	117	305	
s1m1	(-)	1	5	7.28/0.03
s1m2	(-)	-	6	(2.23-23.72)
s1m2	(-)	3	57	

OR: odds ratio; 95% CI=95% confidence interval.

### DISCUSSION

Several authors have demonstrated differences in the geographical distribution of *H. pylori* genotypes, which directly influence the clinical relevance of the strains<sup>(35)</sup>. This study was conducted to increase knowledge regarding the prevalence of *H. pylori* virulence factors in the State of Pará, Northern Brazil. Additionally, infection with this bacterium was related to different gastrointestinal diseases and to the histopathological alterations found in these cases.

The largest number of cases of gastric cancer was due to the study had been conducted at a cancer referral hospital endoscopy service in the region.

A predominance of males was observed among patients with gastric ulcer and gastric cancer. These patients were older than those of the other two groups studied. This higher frequency of gastric ulcers and cancer among men has been associated with lifestyle, since men are more frequently exposed to stress and environmental factors related to habits such as alcohol consumption and smoking<sup>(9)</sup>. The higher incidence of gastric ulcers and cancer in individuals older than 50 years has been related to sequential changes characterized by metaplastic alterations that occur in the gastric mucosa secondary to infection with *H. pylori*<sup>(9, 16)</sup>.

Detection of infection with *H. pylori* by a molecular biology method (PCR) revealed a prevalence of 91% among patients with different gastrointestinal diseases. A high incidence of gastric carcinoma and peptic ulcer, as well as a high prevalence of infection with *H. pylori*, is observed in Brazil, especially in the State of Pará<sup>(1, 18, 36, 37)</sup>.

Some characteristics of *H. pylori* strains have been associated with the progression of infection to more severe disease. In this respect, some genotypes, such as alleles s1 and m1 of the vacA gene and presence of the cagA gene, are considered pathogenicity markers since they are associated with cytotoxin production and the induction of more intense epithelial lesions and inflammatory reactions. In many countries, these genotypes have been associated with the progression of infection to diseases such as peptic ulcer and gastric adenocarcinoma<sup>(1, 3, 10, 12, 14, 22, 31, 38)</sup>.

The *cagA* gene was identified in 85.6% of the patients with gastrointestinal diseases studied. Similar results have been reported in other studies conducted in the State of Pará<sup>(31, 38)</sup> and in other Brazilian states, as well as in other countries<sup>(2, 11, 25, 40)</sup>. When only *H. pylori*-positive patients were compared between the four groups studied, an even stronger association was observed between infection with *cagA*-positive *H. pylori* strains and gastrointestinal diseases. Analysis of the different diseases showed a higher frequency of the *cagA* gene in *H. pylori*-positive patients with duodenal ulcer compared to patients with gastritis, gastric ulcer and gastric cancer. Queiroz et al.<sup>(24)</sup> found that, in Brazil, 95.0% and 62.3% of strains isolated from children with and without duodenal ulcers are *cagA*-positive, respectively. Other studies have reported a higher frequency of the *cagA* gene in *H. pylori*-positive patients with peptic ulcer and gastric cancer<sup>(17, 33, 38)</sup>.

In the present study, 97.6% of the patients were infected with only one type of bacterial strain (mono-infection) and 2.4% were infected with more than one type of strain (mixed infection). In Brazil, the frequency of multiple infections ranges from 3.4% to 17% depending on the populations involved and the region where the study was conducted<sup>(2, 17, 33, 37)</sup>.

Characterization of the *vacA* alleles and investigation of the presence of the *cagA* gene demonstrated that the predominant genotype in this study was s1m1cagA+ (83.4%). Previous studies conducted in the State of Pará reported a higher prevalence of this genotype in patients with different gastrointestinal diseases<sup>(17, 31, 37, 38)</sup>. The same was observed in studies conducted in other Brazilian states such as Belo Horizonte (Minas Gerais)<sup>(2)</sup>, Salvador (Bahia)<sup>(8)</sup>, and Botucatu (São Paulo)<sup>(33)</sup>.

Analysis of the genotypes of *H. pylori* contributes to explain the distribution of gastrointestinal diseases around the world, given the fact that, although the prevalence of *H. pylori* infection is higher in developing countries, some developed countries such as Japan, Portugal and Spain have high frequencies of severe gastrointestinal diseases such as peptic ulcers and gastric cancer. Studies conducted in these countries have associated this fact with the local predominance of *H. pylori* strains carrying genotype s1b/m1cagA+<sup>(15, 21, 35)</sup>.

In the present study, virulent s1m1cagA+ strains were more frequent in patients with gastric ulcer, duodenal ulcer and gastric cancer compared to patients with gastritis. These data indicate a greater association between infection with bacterial strains carrying this genotype (s1m1cagA+) and an increased risk of developing more severe forms of infection. This association is supported by studies conducted in Brazil and in other countries<sup>(7, 8, 17, 33, 37, 39)</sup>.

With respect to the association of different alleles of the *vacA* gene and presence of the *cagA* gene with histopathological findings, we observed that patients infected with s1m1cagA+ strains exhibited a high degree of inflammation and neutrophil activity in the gastric mucosa, as well as an increased risk of developing intestinal metaplasia. These findings agree with the results reported in studies conducted in Brazil and in other countries<sup>(2, 31, 37, 40)</sup> and support the correlation of pathogenic *vacA* and *cagA* genotypes of *H. pylori* with chronic aggression to the mucosa, which is expressed at the morphological level by an inflammatory infiltrate rich in enzymes and free radicals that damage epithelial cells<sup>(33)</sup>.

In conclusion, the results of the present study demonstrate the importance of bacterial factors, such as genotype of the infecting strain, for the pathogenesis of gastrointestinal diseases. These findings are supported by data indicating that patients infected with s1m1cagA+ strains have an increased risk of developing more severe forms of infection. These findings are useful to identify patients who are at a higher risk of developing more severe gastrointestinal diseases, and therefore contribute to the prognosis and treatment of these cases. Further studies establishing the relationship between bacterial, host and environmental factors are necessary to determine what makes a patient to develop duodenal ulcer, gastric ulcer or gastric cancer, since the distribution of the genotypes of infecting strains does not differ between patients with these diseases.

#### Authors' contributions

Vinagre IDF, Queiroz AL and Vinagre RMD: responsible researcher. Silva Jr MR: examination of upper gastrointestinal endoscopy and biopsy. Martins LC: histopathological analysis and genotyping of *H. pylori*.

Vinagre IDF, Queiroz AL, Silva Jr MR, Vinagre RMD, Martins LC. Infecção pelo *Helicobacter pylori* em pacientes com diferentes doenças gastrointestinais do Norte do Brasil. *Arq Gastroenterol*. 2015;52(4):xxx.

**RESUMO - Contexto** - Os mecanismos pelos quais o *H. pylori* produz diferentes quadros patológicos no estômago e no duodeno não são totalmente conhecidos. Considerando a diversidade geográfica relacionada à prevalência dos fatores de virulência desse microrganismo e sua associação com o desenvolvimento de diferentes doenças, vem se intensificando a pesquisa de marcadores de patogenicidade, como o CagA e os alelos do VacA por técnicas moleculares. **Objetivos** - O objetivo desse estudo foi investigar a presença da infecção por *H. pylori*, e a frequência dos diferentes genótipos dessa bactéria em pacientes com doenças gastrointestinais da nossa região, procurando estabelecer sua associação com os achados histopatológicos.

**Métodos** - Em estudo prospectivo, foram coletadas amostras de 554 pacientes com diferentes doenças gastrointestinais (gastrite, úlcera duodenal, úlcera gástrica e câncer gástrico), atendidos em hospital de referência para todo o Estado do Pará, localizado na região metropolitana de Belém. Foram analisados dados obtidos através de questionário epidemiológico, relacionados ao sexo e faixa etária desses pacientes. A presença do *H. pylori* e do genótipo bacteriano foi detectada utilizando a PCR. As biopsias gástricas foram avaliadas histologicamente. **Resultados** - Observou-se uma prevalência de 91% da infecção pelo *H. pylori*, sendo mais frequente nos portadores de úlcera gástrica e câncer gástrico, nos quais houve predomínio do sexo masculino e a idade foi maior que a dos outros dois grupos estudados. O genótipo bacteriano predominante foi o s1m1cagA positivo, sendo mais frequentes entre os pacientes com úlcera gástrica, úlcera duodenal e câncer gástrico. Houve associação significativa das cepas com o genótipo s1m1cagA positivo com maior grau de inflamação, atividade neutrofilica e desenvolvimento de metaplasia intestinal. **Conclusão** - Nosso estudo demonstra a alta incidência da infecção pelo *H. pylori* nos pacientes analisados em nosso meio, especialmente em portadores de úlcera e câncer gástricos. As cepas virulentas s1m1cagA+ foram predominantes e estavam associadas a lesões mais graves.

**DESCRITORES** - *Helicobacter pylori*. Infecções por *Helicobacter*. Gastroenteropatias. Genótipo.

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