The Efficacy of Laboratory Diagnosis of *Helicobacter pylori* Infections in Gastric Biopsy Specimens Is Related to Bacterial Density and *vacA*, *cagA*, and *iceA* Genotypes

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A total of 500 consecutive patients undergoing upper endoscopy were biopsied and tested for *H. pylori* infection by the *Campylobacter*-like organism (CLO) test, culture, histology, and PCR. Serum samples were tested by two different serological assays. Patients were considered *H. pylori* positive if at least two of the four biopsy specimen-based methods yielded positive results. PCR had the highest diagnostic sensitivity (99.4%), followed by histology (92.2%), culture (89.5%), and the CLO test (89.0%). The specificities of all methods were higher than 98%. Of the organisms from the 181 PCR-positive patients, the *vacA* (s and m regions), *cagA*, and *iceA* genotypes were determined by reverse hybridization (line probe assay) or an allele-specific PCR. Organisms that were detected by PCR but that remained undetected by the CLO test were significantly more often *vacA* s1 ($P = 0.006$), m1 ($P = 0.028$), and *cagA* positive ($P = 0.029$) than *vacA* s2, m2, and *cagA* negative, respectively. Organisms that were detected by PCR but that remained undetected by culture or histology more often contained *iceA*1 ($P = 0.034$ and $P = 0.029$, respectively) than *iceA*2. Higher *H. pylori* density was associated with *vacA* s2 ($P = 0.024$), *vacA* m2 ($P = 0.050$), and *cagA*-negative ($P = 0.035$) genotypes. Also, the diagnostic results of the CLO test ($P = 0.001$) and culture ($P = 0.031$) but not those of the PCR ($P = 0.130$) were significantly associated with the *H. pylori* density. The rate of detection by the four biopsy specimen-based tests was lower for patients who used proton pump inhibitors, but this was independent of the *H. pylori* genotypes. These observations may be explained by different bacterial densities, as established by the distinct genotypes of *H. pylori*, and confirm that the biologies of strains with such genotypes are considerably different.

Helicobacter pylori is a gram-negative bacterium involved in the pathogenesis of peptic ulcer disease, gastric carcinoma, and lymphoma. The ecological niche of *H. pylori* is the human stomach, where it establishes long-term colonization of the gastric mucosa (6). The bacterium has a worldwide distribution, and the prevalence ranges from 25% in developed countries to more than 90% in developing areas, but not all infected individuals develop disease.

Diagnosis of *H. pylori* infections can be performed by various methods. Noninvasive methods include serological assays and urea breath tests. When patients are examined by upper endoscopy, an invasive procedure, diagnostic methods can be performed with gastric biopsy specimens, including rapid urease assays (such as the *Campylobacter*-like organism [CLO] test), culture of the bacterium, histological examination, and PCR.

Several *H. pylori* genes that are related to the risk of disease have been identified (2, 5, 13, 15). The cytotoxin-associated gene (*cagA*) is a marker for a genomic pathogenicity (*cag*) island of about 40 kbp whose presence is associated with a more severe clinical outcome (1, 8). This *cag* island contains genes encoding proteins that enhance the virulence of the strain, for example, by increasing cytokine production by host cells (21). A cytotoxin that injures epithelial cells is encoded by *vacA* (9, 11, 12, 14, 18–20). *vacA* is present in all *H. pylori* strains and contains at least two variable parts (3, 20). The

vacA s region (which encodes the signal peptide) exists as s1 or s2 allelic types. Among type s1 strains, subtypes s1a, s1b, and s1c have been identified (24). The m (middle) region occurs as the m1 or the m2 allelic type. Among type m2, two subtypes have been identified, designated m2a and m2b. Production of the vacuolating cytotoxin is related to the mosaic structure of *vacA*. In general, type s1 m1 and type s1 m2 strains produce high and moderate levels of toxin, respectively, while s2 m2 strains show little or no vacuolating toxin activity (3). Since most *vacA* s1 strains are *cagA* positive (3, 10), the two markers are closely related. Among U.S. and European strains, the particular *vacA* s m genotype is a marker of the pathogenicity of an individual strain, since in vitro production of the cytotoxin, in vivo epithelial damage, and peptic ulcer disease all are related to the *vacA* genotype (2, 4, 23). The recently discovered *iceA* (induced by contact with the epithelium) gene exists in two allelic forms, *iceA*1 and *iceA*2 (17). *iceA*1 is upregulated upon contact of *H. pylori* with the gastric epithelium (17) and is a marker for peptic ulcer disease (25).

The aim of the present study was to investigate the relationships between the diagnostic efficacies of several biopsy-based methods and the genotypes of the virulence-associated *vacA*, *cagA*, and *iceA* genes of *H. pylori* in a large group of patients undergoing upper endoscopy.

MATERIALS AND METHODS

Patients. A total of 500 consecutive patients who visited the R. de Graaf Hospital for upper endoscopy were enrolled in the study. Informed consent was obtained from all patients, and all clinical specimens were tested under code.

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Clinical specimens. Upper endoscopy was performed and multiple gastric biopsy specimens were taken from the antrum and the corpus. The biopsy specimens were transported in thioglycolate buffer (2% thioglycolate supplemented with 0.005% hemin, 0.05% L-cysteine hydrochloride, 0.02% L-glutamine, 0.0008% β -nicotinamide adenine dinucleotide monohydrate, 0.0004% thiamine, and 0.001% vitamin K).

One biopsy specimen from the antrum was used for the CLO test. If proton pump inhibitors were used, a biopsy from the corpus was also tested by the CLO test. Sets of two biopsy specimens (one biopsy specimen from the antrum and one from the corpus) were used for culture, histology, and PCR.

A blood sample was obtained from each patient after endoscopy. Serum was prepared within 3 h and was stored at -20°C .

CLO test. The CLO test (Tri-Med) was performed according to the manufacturer's instructions, and the results were interpreted after 24 h.

Culture of *H. pylori*. Freshly retrieved biopsy specimens were transported to the laboratory for culture on the same day. *H. pylori* was cultured by rubbing gastric biopsy specimens onto Columbia agar supplemented with lysed horse blood and Skirrow's supplement (containing vancomycin, trimethoprim, and polymyxin B). The plates were incubated under microaerobic conditions (90% CO_2 , 5% O_2 , 5% N_2) at 35°C for 4 to 7 days. Suspected colonies were tested by gram staining, as well as for urease, oxidase, and catalase activities.

Histopathological examinations. Biopsy specimens for histopathological examinations were fixed in buffered 4% formalin overnight and were embedded in paraffin. Two 4- μm sections were stained with hematoxylin-eosin and one section was stained by the modified Giemsa procedure and examined. The slides were microscopically examined by using a high power (magnification, $\times 400$), and at least five high-power fields were examined. If *H. pylori* was observed, the bacterial density was scored semiquantitatively on an ordinal scale (ranging from 0 to 3) by a single pathologist. All examinations were performed under code.

Detection of *H. pylori* by PCR and genotyping of virulence genes. Biopsy specimens for PCR were frozen in thioglycolate buffer at -80°C within 4 h after the endoscopy. The biopsy specimens were homogenized with a sterile micropestle, and DNA was isolated by the method of Boom et al. (7) and eluted in 100 μl of Tris-HCl (pH 8.3)–10 mM EDTA. The presence of *H. pylori* was detected by multiplex PCR aimed at the *vacA* s and m regions and *cagA* as described previously (24). Amplimers carrying a biotin moiety at the 5' terminus of all reverse primers were detected in a microtiter well-based DNA hybridization assay.

Microtiter wells were precoated with streptavidin. Ten microliters of the PCR product was mixed with amplimer dilution buffer (1 $\times$ SSC [1 $\times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 0.1% Tween 20, 0.004% phenol red). After incubation at 42°C for 30 min, the wells were washed three times with 400 μl of washing solution (1 $\times$ SSC, 0.1% Tween 20). The captured PCR products were denatured by addition of 100 μl of 0.1 M NaOH into the well, and the plate was incubated for 5 min at room temperature. The fluid, which contained the unbiotinylated eluted strand, was removed. Subsequently, 100 μl of hybridization solution (containing 1 $\times$ SSC, 0.1% Tween 20, 0.004% phenol red, and digoxigenin-labelled *vacA*-specific oligonucleotide probes) was added to the well, and the plate was incubated for 45 min at 42°C . After washing the wells three times with washing solution, 100 μl of anti-digoxigenin-peroxidase conjugate (Boehringer Mannheim) per ml was added, and the plate was incubated for 15 min at 42°C . The unbound conjugate was removed by washing the wells five times with washing solution, and 100 μl of substrate solution (containing 0.04% tetramethylbenzidine [TMB] substrate) was added to the wells. After incubation for 15 min at room temperature, the reaction was stopped by addition of 100 μl of 0.5 M sulfuric acid. The optical density of the wells was read at 450 nm in a microtiter plate reader and was compared to the optical densities of negative, borderline, and positive control samples.

vacA and *cagA* genotypes were determined by reverse hybridization of multiplex PCR products by a line probe assay (LiPA), as described previously (23, 24). *iceA* genotypes were determined by separate *iceA1*- and *iceA2*-specific PCRs as described previously (25).

Serological assays. To determine whether immunoglobulin G (IgG) antibodies directed to *H. pylori* were present, two different enzyme immunoassays (EIAs) were used. The first EIA was the Pyloriset EIA-G (Orion Diagnostica, Espoo, Finland), and the second EIA was a prototype assay, the INNOTEST *H. pylori* IgG (Innogenetics, Ghent, Belgium). Both kits were used according to the manufacturers' instructions.

RESULTS

A total of 500 consecutive patients undergoing gastroscopy entered the study, and biopsy specimens as well as serum samples were obtained. These unselected patients had a variety of clinical symptoms such as gastritis, gastric ulcer, duodenal ulcer, and reflux esophagitis. Gastric biopsy specimens were used to determine the presence of *H. pylori* by the CLO test, culture, histopathological examination, and PCR. A complete

set of results obtained by all four methods was available for 450 patients.

The CLO test was performed with samples from 472 patients and yielded positive results for 151 (32.0%) of the patients. Culture of *H. pylori* was positive for 148 (30.9%) of 479 patients tested. *H. pylori* was observed by histological examination in 155 (31.6%) of the 491 specimens examined. PCR yielded positive results for 181 (36.7%) of the 493 biopsy specimens tested. As a "gold standard," patients were considered to be *H. pylori* infected if at least two of the four biopsy specimen-based methods (CLO test, culture, histology, and PCR) yielded positive results. On the basis of this gold standard, a total of 168 of the patients were *H. pylori* positive. The performance of each of the four biopsy specimen-based methods compared to that of the gold standard was assessed separately. In addition, serological results are provided for samples with discrepant results.

CLO test. Compared to the gold standard, 18 patients had false-negative results by the CLO test. Sera were available from 17 of these patients, and all were positive for anti-*H. pylori* IgG antibodies by both EIAs. Six patients were CLO positive but were negative by culture, histology, and PCR. Five of these patients were also seronegative, and one was borderline positive by only one of the two serological assays. Therefore, at least five patients should be considered to have false-positive CLO test results.

Culture. Eighteen patients were positive according to the gold standard but false negative by culture. Sera were available from 17 of these patients, and antibodies were detected in all patients. Three patients were exclusively positive by culture and were all seronegative, and thus, the results for these patients should be considered false positive.

Histological examination. Thirteen samples were false negative by histology compared to the results of the gold standard. Sera were available from 12 patients, and all were seropositive. Two biopsy specimens were scored positive by histological examination only, and one of these was seronegative, whereas the other one was borderline positive only by one of the serological assays.

PCR. Only one sample was false negative by PCR compared to the results of the gold standard. A total of 14 patients were positive only by PCR but negative by the CLO test, culture, and histology. Of these 14 patients, 11 were seropositive by both EIAs. One sample was positive by only one of the serological assays, and two samples were seronegative by both EIAs. Therefore, at least 11 of the 14 patients may not be considered to have had false-positive results but their specimens probably contained very small amounts of bacteria, amounts that were below the detection limits of the CLO test, culture, and histological examination. The results of each of the diagnostic methods were compared to those of the gold standard. The sensitivity, specificity, and positive and negative predictive values were calculated for each of the methods, as shown in Table 1. It should be noted that the serological methods were also evaluated on the basis of the biopsy specimen-derived gold standard.

***H. pylori vacA*, *cagA*, and *iceA* genotypes.** The *vacA*, *cagA*, and *iceA* genotypes were determined for the *H. pylori* strains in all 181 PCR-positive biopsy specimens (Table 2). *vacA* s1 was observed in 132 patients, and of the organisms from these patients, 116 (87.9%) were subtype s1a, 14 (10.6%) were subtype s1b, and 2 (1.5%) were subtype s1c. Thirty-four samples contained *H. pylori vacA* type s2. Seventeen samples contained multiple *vacA* genotypes, indicating the presence of more than one *H. pylori* strain.

Among the 181 PCR-positive samples, 127 (70.2%) were

TABLE 1. Sensitivities and specificities of the different diagnostic methods for detection of *H. pylori* infection^a

Method	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Biopsy specimen based				
CLO test	89.0	98.1	96.0	94.4
Culture	89.5	99.1	97.8	94.6
Histology	92.2	99.7	99.3	96.1
PCR	99.4	95.6/99.1 ^b	98.2	99.7
Serum specimen based				
INNOTEST <i>H. pylori</i> IgG	98.1	83.2	74.6	98.9
Pyloriset EIA-G	96.3	84.2	75.2	97.8

^a All calculations (including those for the serological assays) are based on the biopsy specimen-based gold standard (at least two of the four biopsy specimen-based methods should yield positive results).

^b When the serological results are also taken into account, of the 14 PCR-positive specimens, at least 11 were also seropositive.

cagA positive, 89 (49.1%) samples contained *iceA1*, and 76 (42.0%) contained *iceA2*. Twelve samples (6.6%) contained both *iceA* alleles, and three of these also contained multiple *vacA* types. The *iceA* genotype could not be determined for four (2.2%) of the PCR-positive samples.

The combination of the *vacA* s- and m-regions, *cagA*, and *iceA* genotypes was also investigated. Of the 16 possible genotypes, the s1 m1 *cagA* positive *iceA1* (24.3%) genotype was the most prevalent. As expected, no samples containing a *vacA* s2 m1 genotype were found. The nonrandom distribution of the genotypic combinations confirmed the associations between the individual *vacA*, *cagA*, and *iceA* genotypes. *cagA*-positive strains are frequently *vacA* s1 ($P < 0.001$), *vacA* m1 ($P < 0.001$), and *iceA1* ($P = 0.018$). The *iceA1* genotype is also associated with *vacA* s1 ($P < 0.001$) and *vacA* m1 ($P < 0.001$).

Association between *vacA*, *cagA*, and *iceA* genotypes and efficacies of CLO test, culture, and histology. Since the diagnostic sensitivity of the PCR was greater than those of the CLO test, culture, and histology, the relationships between efficacy

of diagnosis and *H. pylori* genotypes were further analyzed. Only 144 of the 181 PCR-positive samples were positive by the CLO test. The distribution of the *vacA*, *cagA*, and *iceA* genotypes between the group of samples found to be positive by the CLO test and those not found to be positive by the CLO test was compared, and the results are shown in Table 2. Samples that remained negative by the CLO test were more frequently *vacA* s1, *vacA* m1, *cagA* positive, and, to a lesser extent, *iceA1* than *vacA* s2, m2, *cagA* negative, and *iceA2*, respectively. PCR-positive samples that remained negative by culture or histology did not show significant differences for the *vacA* s, *vacA* m, and *cagA* genotypes but were more frequently *iceA1* than *iceA2*.

These differences were also observed when the combinations of genotypes were considered. Samples with the *vacA* s1 m1 *cagA* positive *iceA1* genotype remained negative by the CLO test more frequently than samples with the *vacA* s2 m2 *cagA* negative *iceA2* genotype ($P = 0.002$). Similarly, samples containing the *vacA* s1 m1 *cagA* positive *iceA1* genotype more often remained negative by culture ($P = 0.02$) or histology ($P = 0.01$) than those containing the s1 m1 *cagA* positive *iceA2* genotype.

Associations between the *vacA*, *cagA*, and *iceA* genotypes and the *H. pylori* density. The presence of *H. pylori* in gastric microscopic sections was quantified for samples that were gold standard positive on an arbitrary scale of 0 to 3 by a single pathologist, and the results are shown in Table 3. Patients with higher *H. pylori* densities were significantly more often found to be positive by the CLO test and culture. In contrast, the efficacy of *H. pylori* detection by PCR was not significantly associated with bacterial load. *H. pylori* density was significantly higher in patients infected with *vacA* s2 strains than in those infected with *vacA* s1 strains. Similarly, higher densities were observed in patients infected with strains with the *vacA* m2 or *cagA*-negative genotype than in patients infected with *vacA* m1 or *cagA*-positive strains, respectively. The *iceA* status was not significantly associated with bacterial load.

Comparison of serological assays. Two different immunoassays were used to determine the presence of anti-*Helicobacter* IgG antibodies. Of the 322 patients who were negative by the

TABLE 2. Correlation between detection of *H. pylori* infection by CLO test, culture, and histology and *H. pylori vacA*, *cagA*, and *iceA* genotype

Genotype	CLO test (n = 175)			Culture (n = 176)			Histology (n = 178)		
	No. of specimens		P value ^a	No. of specimens		P value	No. of specimens		P value
	Positive	Negative		Positive	Negative		Positive	Negative	
<i>vacA</i>									
<i>vacA</i> s1	98	30	0.006	100	28	0.23	107	22	0.10
<i>vacA</i> s2	32	1		29	4		32	2	
<i>vacA</i> s multiple	14	0		15	0		13	2	
<i>vacA</i> m1	59	20	0.028	66	12	0.33	66	14	0.44
<i>vacA</i> m2	81	11		74	20		83	12	
<i>vacA</i> m multiple	4	0		4	0		3	0	
<i>cagA</i>									
<i>cagA</i> positive	96	27	0.029	102	21	0.67	104	21	0.09
<i>cagA</i> negative	48	4		42	11		43	3	
<i>iceA</i>									
<i>iceA</i> negative	2	2		1	3		3	0	
<i>iceA1</i>	65	20	0.066	68	20	0.034	71	18	0.029
<i>iceA2</i>	66	9		65	7		68	6	
<i>iceA</i> multiple	11	0		10	2		10	2	

^a Calculated by Fisher's exact test; specimens containing multiple genotypes were excluded from the analysis.

TABLE 3. Associations between *H. pylori* density, diagnostic results, and genotypes among gold standard-positive specimens

Test and result or genotype	No. of specimens with the following <i>H. pylori</i> density score:				P value ^a
	0	1	2	3	
CLO test					
Positive	6	26	38	66	0.002
Negative	3	5	4	2	
Culture					
Positive	5	28	38	61	0.031
Negative	5	3	5	5	
PCR					
Positive	11	31	43	68	0.130
Negative	0	0	0	1	
<i>vacA</i> s					
<i>vacA</i> s1	10	25	30	43	0.024
<i>vacA</i> s2	1	2	11	18	
<i>vacA</i> m					
<i>vacA</i> m1	5	19	17	25	0.050
<i>vacA</i> m2	6	10	25	42	
<i>cagA</i>					
Positive	8	28	26	43	0.035
Negative	3	3	17	26	
<i>iceA</i>					
<i>iceA</i> 1	8	15	21	27	0.135
<i>iceA</i> 2	3	14	18	34	

^a Calculated by the Mann-Whitney U test. Specimens containing multiple genotypes were excluded from the analysis.

gold standard, a considerable number were found to be seropositive (54 by the INNOTEST *H. pylori* IgG test and 51 by the Pyloriset EIA-G). Discrepant serological results were obtained for 34 (7.0%) of the 483 serum samples tested by both assays. By using the biopsy specimen-based gold standard, the specificities and sensitivities of the INNOTEST *H. pylori* IgG test (83.2 and 98.1%, respectively) and the Pyloriset EIA-G (84.2 and 96.3%, respectively) were calculated (Table 1).

Among patients who were serologically positive, individuals who used proton pump inhibitors were significantly more frequently negative by the gold standard, indicating that overall growth of *H. pylori* is probably inhibited in those patients (data not shown). However, this inhibition was not significantly associated with any of the *H. pylori* genotypes.

DISCUSSION

Accurate diagnosis of *H. pylori* infection is an important aspect of the management of patients with gastrointestinal symptoms. Usually, a combination of different methods is used, depending on the availability of different methods in routine clinical practice. Currently, most diagnoses are based on analysis of gastric biopsy specimens obtained by upper endoscopy.

The present study analyzed a cohort of consecutive patients who underwent upper endoscopy. Most patients were born in The Netherlands, and this is reflected by the high prevalence of strains containing the *vacA* s1a genotype, confirming earlier data on the geographic distributions of *H. pylori* variants (22). Subtype s1c, which is highly prevalent in East Asia but which is

rare in other parts of the world, was observed in only two patients, and one of those patients was born in Vietnam.

A patient was considered *H. pylori* infected if at least two of the four biopsy specimen-based assays yielded positive results. On the basis of this gold standard, the calculated specificities of all four biopsy specimen-based methods were greater than 98%. However, the sensitivities varied considerably, ranging from 89% for the CLO test and culture to 92% for histology and 99% for PCR. Since the CLO test measures the urease activity of *H. pylori* in the gastric mucosa, the sensitivity of the CLO test depends on the number of bacteria and their total level of urease production. In vitro culture of *H. pylori* depends on the amount and viability of the cells. Since *H. pylori* is sensitive to oxygen, the efficacy of *H. pylori* culture also depends on the time lag between the time of sampling by biopsy and the start of culture of the sample under microaerobic conditions. Histological detection is based on visual observation of stained bacteria. PCR permits specific amplification of bacterial DNA from the biopsy specimen. Both histology and PCR are therefore unrelated to any activity or viability of the cells. The high sensitivity of the PCR was confirmed by the serological assays. At least 11 of the 14 patients who were exclusively positive by PCR were also seropositive, indicating past or present *H. pylori* infection. In contrast, patients who were exclusively positive by the CLO test, culture, or histology were almost all seronegative. These data indicate that PCR offers the most sensitive and specific biopsy specimen-based method for diagnosis of *H. pylori* infection. Multiplex PCR can be performed directly with gastric biopsy specimens, and its products can be rapidly detected in a microtiter-format hybridization assay. Subsequently, the same PCR products can be used to determine the *vacA*, *cagA*, and *iceA* status by reverse hybridization by a line probe assay (23, 24).

Two different immunoassays were used to measure anti-*Helicobacter* IgG titers in serum. The performance characteristics (specificities and sensitivities) of both assays were highly similar. A considerable number of patients were seropositive, while all biopsy specimen-based methods yielded negative results. It should be noted that serological methods provide only indirect evidence of infection and cannot discriminate between present and past infections. These patients could have been infected in the past, but data on previous use of antibiotics were not available. However, it appeared that these patients often used proton pump inhibitors, indicating that this results in a reduction in the bacterial density in the stomach while leaving antibody titers unaffected. Thus, diagnosis of *H. pylori* infection by a biopsy specimen-based test can be insufficient for patients who use proton pump inhibitors, and a biopsy specimen-based test should be combined with a serological assay.

The present study investigated the relationships between the *vacA*, *cagA*, and *iceA* genotypes of *H. pylori* and diagnosis of infection. Compared to PCR, the sensitivities of the CLO test, culture, and histology were limited. More importantly, the diagnostic efficacy was associated with *H. pylori* genotypes. The CLO test is related to the *vacA* s region and *cagA* genotypes, whereas culture and histopathology are significantly associated with *iceA* genotypes. These observations may be explained by the finding that there are also significant relations between the *H. pylori* genotypes and the bacterial density in the stomach. Although the histological assessment of *H. pylori* density in a single biopsy specimen on an ordinal semiquantitative scale is not highly accurate, some trends could be observed. Certain genotypic variants establish higher levels of colonization of the gastric mucosa and are therefore easier to detect by histological assessment, the CLO test, and culture. Interestingly, strains with the *vacA* s1, *vacA* m1, *iceA*1, or *cagA*-positive

genotype, which are considered the more virulent, appear to reach lower densities than *vacA* s2, *vacA* m2, *iceA*2, or *cagA*-negative strains. Therefore, it seems unlikely that the increased pathogenicity of the *vacA* s1, *vacA* m1, *iceA*1, or *cagA*-positive *H. pylori* strains is directly proportional to the bacterial load.

These data are also in agreement with earlier observations showing that *vacA* s2 or *cagA*-negative strains (26, 27), as well as strains that reach a higher bacterial density (16), are more resistant to antibiotic treatment.

In conclusion, specific *H. pylori* lineages establish relatively low bacterial densities and are therefore more difficult to detect by the CLO test and culture. Conversely, other *H. pylori* genotypes reach higher densities and rarely yield false-negative results. These findings confirm that there are significant biological differences between the distinct genotypic variants of *H. pylori*, and these differences may have implications for the effective and reliable detection of this important human pathogen.

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