Virulence genes of *Helicobacter pylori* in the Dominican Republic

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Although the incidence of gastric cancer in the Dominican Republic is not high, the disease remains a significant health problem. We first conducted a detailed analysis of *Helicobacter pylori* status in the Dominican Republic. In total, 158 patients (103 females and 55 males; mean age 47.1 ± 16.2 years) were recruited. The status of *H. pylori* infection was determined based on four tests: rapid urease test, culture test, histological test and immunohistochemistry. The status of *cagA* and *vacA* genotypes in *H. pylori* was examined using PCR and gene sequencing. The overall prevalence of *H. pylori* infection was 58.9 %. No relationship was found between the *H. pylori* infection rate and the age range of 17–91 years. Even in the youngest group (patients aged 29 years), the *H. pylori* infection rate was 62.5 %. Peptic ulcer was found in 23 patients and gastric cancer was found in one patient. The *H. pylori* infection rate in patients with peptic ulcer was significantly higher than that in patients with gastritis (82.6 versus 54.5 %, *P*<0.01). The *cagA*-positive/*vacA* s1m1 genotype was the most prevalent (43/64, 67.2 %). Compared with *H. pylori*-negative patients, *H. pylori*-positive patients showed more severe gastritis. Furthermore, the presence of *cagA* was related to the presence of more severe gastritis. All *CagA*-positive strains had Western-type *CagA*. In conclusion, we found that *H. pylori* infection is a risk factor for peptic ulcer in the Dominican Republic. Patients with *cagA*-positive *H. pylori* could be at higher risk for severe inflammation and atrophy.

INTRODUCTION

*Helicobacter pylori* is a spiral, Gram-negative bacterium that chronically colonizes the human stomach and is currently recognized as playing a causative role in the pathogenesis of various gastroduodenal diseases, including gastritis, peptic ulcer, gastric cancer and mucosa-associated lymphoid tissue lymphoma (Peek & Blaser, 2002; Suerbaum & Michetti, 2002). Gastric cancer remains a significant health problem, although its incidence greatly varies geographically. Countries can be categorized as high risk (e.g. Japan), intermediate risk (e.g. Vietnam) or low risk (e.g. the United States) for gastric cancer based on the age-standardized...
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virulence genes of

and immunohistochemistry (IHC). We also identified the
multiple tests: rapid urease test, culture test, histological test
infection rate of

2007; Leung

2013; Miki, 2011). Furthermore, previous studies of the
prevalence of chronic atrophic gastritis in the Dominican
Republic were undertaken based on pepsinogen measure-
ment. The ASRs of gastric cancer in Caribbean countries are reportedly <10: 100 000 year−1 (http://globocan.
arc.fr/). The ASR of gastric cancer in the Dominican Republic is reportedly 7.3: 100 000 year−1 (http://globocan.arc.fr/).
Although the prevalence of H. pylori infection has been determined in several countries with different socioeconomic,
cultural and racial makeups (Azevedo et al., 2009;
Goh et al., 2011; Tonkic et al., 2012), the prevalence of H.
pylori infection in the Dominican Republic has not yet been
investigated thoroughly. A previous study reported that the
age-adjusted seroprevalence of H. pylori infection in the
Dominican Republic was 62.1 % (Aoki et al., 2004). This
finding suggested that the low virulence of H. pylori in the
Dominican Republic contributed to the low incidence of
gastric cancer. However, serological H. pylori antibody titres
varied greatly depending on the test kit used (Burucoa et al.,
2013; Miki, 2011). Furthermore, previous studies of the
prevalence of chronic atrophic gastritis in the Dominican
Republic were undertaken based on pepsinogen measure-
ments, not histology (Aoki et al., 2004, 2005). Variable cut-
off values for pepsinogen I and the pepsinogen I/II ratio
were applied in previous studies, and might have affected
the sensitivity and specificity of the results (Brenner et al.,
2007; Leung et al., 2008). In this study, we determined the
infection rate of H. pylori in the Dominican Republic using
multiple tests: rapid urease test, culture test, histological test
and immunohistochemistry (IHC). We also identified the
virulence genes of H. pylori in the Dominican Republic.

METHODS

Study population. We recruited 158 patients with dyspeptic
symptoms (103 females and 55 males; mean age 47.1 ± 16.2 years,
range 17–91 years) at the Digestive Disease Center, Dr Luis E. Aybar
Health and Hygiene City, Santo Domingo, Dominican Republic, in
February 2011. Patients with a history of partial gastric resection or
previous treatment for H. pylori infection were excluded. Written
informed consent was obtained from all participants, and the protocol
was approved by the ethics committees of Dr Luis E. Aybar Health and
Hygiene City and Oita University Faculty of Medicine, Japan.

During each endoscopy session, four gastric biopsy specimens were
obtained (three from the antrum and one from the corpus). The
antrum specimens were used for H. pylori culture, rapid urease test
and histological examination. The corpus specimen was used for
histological examination. Peptic ulcer and gastric cancer were
identified using endoscopy. Gastritis was diagnosed in the absence
of peptic ulcer or gastric malignancy.

Status of H. pylori infection. To maximize the diagnostic accuracy,
we used four methods for the diagnosis of H. pylori infection: rapid
urease test, culture test, histological test and IHC. The CLOtest (Kimberly
Clark Ballard Medical Products) was used as a rapid urease test to detect
the presence of H. pylori urease. H. pylori culture was performed as
described previously (Yamaoka et al., 1998b). For histology, all biopsy
specimens were fixed in 10 % buffered formalin for 24 h and then
embedded in paraffin. Serial sections were stained with haematoxylin–
eosin and May–Giemsa. The degree of bacterial load was classified into
groups according to the updated Sydney System (Dixon et al., 1996).
Grade ≥ 1 bacterial load was defined as H. pylori-positive. Patients were
considered H. pylori-negative when all four tests were negative, whereas
the H. pylori-positive status required at least one positive result.

IHC. IHC was performed as described previously (Uchida et al.,
2007). Briefly, after antigen retrieval and inactivation of endogenous
peroxidase activity, tissue sections were incubated with anti-H. pylori
antibody (Dako) overnight at 4 °C. After washing, the sections were
incubated with biotinylated goat anti-rabbit IgG (Nichirei), followed
by incubation with a solution of avidin-conjugated horseradish
peroxidase (Vector). In all cases, we performed Giemsa staining using
a serial section to identify the presence of H. pylori. If the H. pylori
identified by Giemsa staining was positively immunostained, we
considered the patient to be H. pylori-positive.

Staging for gastritis. The degree of gastritis was classified using four
grades according to the updated Sydney System (Dixon et al., 1996);
samples of grade ≥ 1 were considered atrophy-positive, according to
a previous report (Bornschein et al., 2012). In addition, the gastritis
stage was assessed based on the severity and topographic locations
(antrum and corpus) according to the Operative Link on Gastritis
Assessment (OLGA) system (Rugge et al., 2005, 2007).

Isolation and genotyping of H. pylori. H. pylori DNA was extracted from
H. pylori cultured to confluence on plates using a commercially
available kit (Qiagen). The status of cagA was determined with PCR for
a conserved region of cagA and for direct sequencing as described
previously (Yamaoka et al., 2000). The cagA genotype (East-Asian type
and Western type) was confirmed by sequencing the PCR products
as described previously (Xia et al., 2009). We performed vacA genotyping
(s1, s2, m1 and m2) as described previously (Atherton et al., 1995;
Yamaoka et al., 1999a). The PCR products were analysed by gel
electrophoresis using 1.5 % agarose gel containing ethidium bromide.

Statistical analysis. Data were analysed using SPSS version 19
(SPSS). Statistical evaluation was performed with the χ2 test
to compare discrete variables, and with the Mann–Whitney U test and
the t-test to compare continuous variables. To match age and sex, we used
multiple backward stepwise logistic regression analyses to examine
the associations of peptic ulcer with the main predictor variables.
Predictor variables for peptic ulcer consisted of age, sex and H. pylori
status. For each variable, the odds ratio and 95 % confidence interval
were calculated. A two-tailed P<0.05 was considered significant.

RESULTS

Prevalence of H. pylori infection in the Dominican Republic

Table 1 shows the H. pylori-positive rate for each test. The histology and IHC results matched completely, and are
shown as 'histological examination'. Compared with the positive rate of the culture, that of histological examination was significantly higher ($P < 0.01$). Although not statistically significant, the rapid urease test showed a positive rate lower than that of the histological examination. Overall, the prevalence of $H. pylori$ infection in the Dominican Republic was 58.9% (93/158). Fig. 1 shows the prevalence of $H. pylori$ infection in various age groups. No relationship was found between $H. pylori$ infection rate and age ranging from 17 to 91 years ($P = 0.81$).

**Endoscopic findings and $H. pylori$ infection rate**

Gastritis was the most common finding (134/158, 84.8%). Among 134 patients with gastritis, four had gastric polyps and one had a submucosal tumour. Gastric and duodenal ulcers were found in 13 (8.2%) and 10 (6.3%) patients, respectively. Gastric cancer was found in one patient (0.6%). Fig. 2 shows the prevalence of $H. pylori$ infection in patients with gastritis and peptic ulcer. A high infection rate was detected among patients with gastric ulcer (84.6%) and duodenal ulcer (80.0%), whereas 52.8% of the patients with gastritis were $H. pylori$-positive. When gastric and duodenal ulcers were defined as peptic ulcers, the prevalence of $H. pylori$ infection in peptic ulcer was significantly higher than that in gastritis (82.6 versus 54.5%, $P = 0.008$). Multiple logistic analysis after adjustment for age and gender showed that $H. pylori$ positivity was significantly associated with peptic ulcer (odds ratio 3.96; 95% confidence interval 1.28–12.29). The patient with gastric cancer was infected with $H. pylori$.

**Virulence genes of $H. pylori$**

DNA was successfully extracted from 64 of 68 cultured strains and virulence genes were examined in these 64 strains (47 from patients with gastritis, 16 from patients with peptic ulcer and one from the patient with gastric cancer). The distributions of the $cagA$ and $vacA$ genotypes in the Dominican Republic are shown in Table 2. The prevalence of $cagA$ was 75.0% (48/64). The $vacA$ s1 genotype was the most common (48/64, 75.0%). The prevalence of the $vacA$ m1 genotype was 71.9% (46/64). Among the genotypes combining the $vacA$ s and m regions, 44 (68.8%) were $s1m1$, four (6.3%) were $s1m2$, two (3.1%) were $s2m1$ and 14 (21.9%) were $s2m2$. Among the genotypes combining $cagA$ and $vacA$, the $cagA$-positive/$vacA$ s1m1 genotype was the most prevalent (43/64, 67.2%). The types of $cagA$ and $vacA$ were compared between patients with gastritis and peptic ulcer (Table 2). No differences in the

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**Table 1.** Prevalence [$n$ (%)] of $H. pylori$ infection determined by diagnostic tests

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Total no. subjects</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>17–29</td>
<td>24</td>
<td>158</td>
</tr>
<tr>
<td>30–39</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>40–49</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>50–59</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>60–91</td>
<td>36</td>
<td></td>
</tr>
</tbody>
</table>

| Rapid urease test | 13 (54.2) | 14 (50.0) | 22 (55.0) | 16 (53.3) | 13 (36.1) | 78 (49.4) |
| Culture         | 10 (41.7)  | 14 (50.0) | 21 (52.5) | 12 (40.0) | 11 (30.6) | 68 (43.0) |
| Histological examination | 14 (58.3) | 17 (60.7) | 24 (60.0) | 16 (53.3) | 18 (50.0) | 89 (56.3) |
| Final           | 15 (62.5)  | 17 (60.7) | 25 (62.5) | 18 (60.0) | 18 (50.0) | 93 (58.9) |

**Fig. 1.** Prevalence of $H. pylori$ infection in various age groups in the Dominican Republic. $H. pylori$ infection was examined using four methods: rapid urease test, culture test, histological test and IHC. Patients were considered $H. pylori$-negative when all four tests were negative, whereas the $H. pylori$-positive status required at least one positive test result.

**Fig. 2.** Prevalence of $H. pylori$ infection in patients with gastritis and peptic ulcer.
cagA and vacA genotypes were found between the gastritis and peptic ulcer groups (all P>0.05).

The CagA types derived from the cagA genotypes by gene sequencing were also examined. Thirty-nine strains were sequenced successfully for cagA. All 39 showed Western-type CagA. Among the 39 Western-type CagA strains, 33 (84.6%) were ABC type. Strains with multiple C segments (i.e. ABCC) were found in three patients (two with gastritis and one with gastric ulcer). Other types of CagA (AB, AABC and ABBC) were found in one patient each.

Table 2. Virulence genes [n (%)] of H. pylori in the Dominican Republic

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Gastritis</th>
<th>Peptic ulcer</th>
<th>Gastric cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>64</td>
<td>47</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>cagA (+)</td>
<td>48 (75.0%)</td>
<td>36 (76.6)</td>
<td>12 (75.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>cagA (-)</td>
<td>16 (25.0)</td>
<td>11 (23.4)</td>
<td>4 (25.0)</td>
<td>1 (100.0)</td>
</tr>
<tr>
<td>vacA s1</td>
<td>48 (75.0)</td>
<td>36 (76.6)</td>
<td>12 (75.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>vacA s2</td>
<td>16 (25.0)</td>
<td>11 (23.4)</td>
<td>4 (25.0)</td>
<td>1 (100.0)</td>
</tr>
<tr>
<td>vacA m1</td>
<td>46 (71.9)</td>
<td>34 (72.3)</td>
<td>12 (75.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>vacA m2</td>
<td>18 (28.1)</td>
<td>13 (27.7)</td>
<td>4 (25.0)</td>
<td>1 (100.0)</td>
</tr>
<tr>
<td>vacA s1m1</td>
<td>44 (68.8)</td>
<td>33 (70.2)</td>
<td>11 (68.8)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>vacA s1m2</td>
<td>4 (6.3)</td>
<td>3 (6.4)</td>
<td>1 (6.3)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>vacA s2m1</td>
<td>2 (3.1)</td>
<td>1 (2.1)</td>
<td>1 (6.3)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>vacA s2m2</td>
<td>14 (21.9)</td>
<td>10 (21.3)</td>
<td>3 (18.8)</td>
<td>1 (100.0)</td>
</tr>
<tr>
<td>cagA (+) vacA s1m1</td>
<td>43 (67.2)</td>
<td>32 (68.1)</td>
<td>11 (68.8)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>cagA (+) vacA s1m2</td>
<td>3 (4.7)</td>
<td>2 (4.3)</td>
<td>1 (6.3)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>cagA (+) vacA s2m1</td>
<td>1 (1.6)</td>
<td>1 (2.1)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>cagA (+) vacA s2m2</td>
<td>1 (1.6)</td>
<td>1 (2.1)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>cagA (-) vacA s1m1</td>
<td>1 (1.6)</td>
<td>1 (2.1)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>cagA (-) vacA s1m2</td>
<td>1 (1.6)</td>
<td>1 (2.1)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>cagA (-) vacA s2m1</td>
<td>1 (1.6)</td>
<td>0 (0.0)</td>
<td>1 (6.3)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>cagA (-) vacA s2m2</td>
<td>13 (20.3)</td>
<td>9 (19.1)</td>
<td>3 (18.8)</td>
<td>1 (100.0)</td>
</tr>
</tbody>
</table>

Gastric mucosa status

Histological findings classified 35 patients as grade 0 for atrophy in the antrum; 106 patients had grade 1, 17 had grade 2 and none had grade 3. Atrophy in the corpus was classified as grades 0, 1 and 2 for 117, 37 and four patients, respectively. When samples of grade ≥1 were considered atrophy-positive, 123 patients (77.8%) were found to have mucosal atrophy in the antrum, and 41 (25.9%) patients also had mucosal atrophy in both the antrum and the corpus.

The staging of gastritis was also assessed according to the OLGA system. Stage 0 was found in 22.1%, stage I in 64.5% (102/158) and stage II in 13.2% (21/158). Stages III and IV were not found. The differences in histological scores according to the status of H. pylori infection are shown in Table 3. Compared with H. pylori-negative subjects, H. pylori-positive subjects showed significantly greater activity, inflammation and atrophy in both the antrum and the corpus (all P<0.0001). The difference in histological scores according to the status of H. pylori infection is shown in Table 3. Compared with H. pylori-negative subjects, H. pylori-positive subjects showed significantly greater activity, inflammation and atrophy in both the antrum and the corpus (all P<0.0001). The OLGA score in H. pylori-positive subjects was also significantly higher than that in negative patients (1.08±0.55 versus 0.68±0.56, P<0.0001). Eleven and seven subjects had intestinal metaplasia in the antrum and corpus, respectively. The prevalence of intestinal metaplasia in the antrum was not significantly different between H. pylori-positive and H. pylori-negative subjects (8.6 versus 6.1%, P=0.56). Likewise, no differences in the presence of intestinal metaplasia in the corpus were found between H. pylori-positive and H. pylori-negative subjects (5.3 versus 3.0%, P=0.48).

Histological scores according to cagA status are shown in Table 4. Compared with cagA-negative patients, cagA-positive patients had significantly higher scores for atrophy

Table 3. Differences in histological scores according to the status of H. pylori infection

<table>
<thead>
<tr>
<th></th>
<th>H. pylori (+)</th>
<th>H. pylori (-)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antrum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activity</td>
<td>1.33±0.64</td>
<td>0.12±0.37</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Inflammation</td>
<td>1.81±0.63</td>
<td>0.51±0.71</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Atrophy</td>
<td>1.04±0.53</td>
<td>0.66±0.53</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Intestinal metaplasia</td>
<td>0.14±0.52</td>
<td>0.08±0.40</td>
<td>0.33</td>
</tr>
<tr>
<td>Bacterial density</td>
<td>1.58±0.82</td>
<td>0.00±0.00</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Corpus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activity</td>
<td>1.00±0.67</td>
<td>0.11±0.40</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Inflammation</td>
<td>1.26±0.56</td>
<td>0.32±0.58</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Atrophy</td>
<td>0.40±0.55</td>
<td>0.12±0.37</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Intestinal metaplasia</td>
<td>0.10±0.44</td>
<td>0.05±0.27</td>
<td>0.48</td>
</tr>
<tr>
<td>Bacterial density</td>
<td>1.60±0.75</td>
<td>0.00±0.00</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>OLGA score</td>
<td>1.08±0.55</td>
<td>0.68±0.56</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
The World Health Organization and UNICEF have reported that the percentage of improved sanitation facilities in 2011 was 82% in the Dominican Republic. By contrast, that figure was 100% in Japan and the United States, where *H. pylori* infection rates are decreasing (http://www.unicef.org/). Unimproved sanitary conditions in the Dominican Republic may be associated with high *H. pylori* infection rates. Nevertheless, the prevalence of *H. pylori* infection was lowest in the older age group and we reported previously that the prevalence of *H. pylori* infection decreased with age in Bhutan, which is also a developing country (Vilaichone et al., 2013). The decrease in *H. pylori* infection rate with age might be due to frequent antibiotic use in infectious diseases in general, but further studies are necessary to clarify the factors influencing the *H. pylori* infection rate.

Our finding that the prevalence of *H. pylori* in patients with peptic ulcer was significantly higher than that in patients with gastritis was consistent with the findings of previous studies (Kodama et al., 2006). This relationship suggests that *H. pylori* infection is a risk factor for the development of peptic ulcer and gastric cancer in the Dominican Republic. Furthermore, histological scores were higher in *H. pylori*-positive patients than in *H. pylori*-negative patients, consistent with the results of other studies (Kodama et al., 2012b). Successful eradication is effective for the prevention of not only peptic ulcer, but also gastric cancer (Asaka et al., 2010). Additionally, 10-year prospective follow-up studies have shown that successful eradication therapy reduces significantly mucosal inflammation, activity and atrophy (Kodama et al., 2012a, b). Therefore, eradication therapy for *H. pylori* infection can be useful in reducing the occurrence of peptic ulcer and gastric cancer in the Dominican Republic.

We found that 77.8% of subjects had mucosal atrophy in the antrum, and 25.9% subjects also had mucosal atrophy in both the antrum and the corpus in the Dominican Republic. We reported previously that mucosal atrophy was found in the antrum in 91.9% of subjects and in the corpus in 37.7% of subjects in Bhutan, where the incidence of gastric cancer is high (17.2 : 100 000 year−1) (Shiota et al., 2013). Another staging system for gastritis (OLGA) showed that 22.1% of subjects were classified as stage 0, 64.5% as stage I and 13.2% as stage II in the Dominican Republic. Stages III and IV were not observed. On the contrary, ~40% of subjects were classified as stages III and IV in a study of Japanese patients (Satoh et al., 2008). Milder gastritis might be related to the low incidence of gastric cancer in the Dominican Republic despite the high *H. pylori* infection rate.

To our knowledge, this report is the first to reveal the virulence factors of *H. pylori* in the Dominican Republic. The *cagA* gene, which encodes a highly immunogenic protein (CagA), is the most extensively studied *H. pylori* virulence factor (Covacci et al., 1993; Tummuru et al., 1993). Almost all *H. pylori* isolates from East-Asian countries and ~60–80% of isolates from Western countries in the antrum and corpus (P=0.03 and 0.01, respectively). Inflammation scores in the corpus of cagA-positive patients were also significantly higher than in those of cagA-negative patients (P=0.03). Interestingly, none of the 16 cagA-negative patients had intestinal metaplasia in the antrum or corpus. The OLGA score in cagA-positive patients was also significantly higher than that in cagA-negative patients (1.15 ± 0.50 versus 0.81 ± 0.54, P=0.03).

### DISCUSSION

Using a combination of four tests, we determined the prevalence of *H. pylori* in the Dominican Republic to be 58.9%. Similar results have been reported in neighbouring countries, such as Haiti (60.0%) and Jamaica (50.8%) (Hisada et al., 2001; Shak et al., 2011). In contrast with those in developed countries, *H. pylori* infections in the developing world occur earlier in life and with a higher frequency (Goh et al., 2011). The prevalence of *H. pylori* infection was reported to be >70% in developing countries (Calvet et al., 2013; Leja et al., 2012; Porras et al., 2013). The present study showed that the prevalence was high even in young age groups (58.3 % in patients aged ≤29 years) in the Dominican Republic. The high infection rate in the younger age group in our study was consistent with that reported in a study conducted in 2001–2002 (Aoki et al., 2005). Sanitary conditions (e.g. availability of necessary equipment for water and sewage management) are considered important factors in *H. pylori* infection (Goh et al., 2011). Poor sanitary conditions are positively associated with a high rate of *H. pylori* positivity (Dattoli et al., 2010; Strebel et al., 2010). Furthermore, the prevalence of *H. pylori* infection decreased along with improvements in clean public water systems after World War II in Japan (Asaka et al., 1992). The World Health Organization and UNICEF have reported that the

### Table 4. Histological scores according to the status of cagA

<table>
<thead>
<tr>
<th></th>
<th>cagA (+)</th>
<th>cagA (−)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antrum</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activity</td>
<td>1.48 ± 0.61</td>
<td>1.25 ± 0.44</td>
<td>0.20</td>
</tr>
<tr>
<td>Inflammation</td>
<td>1.94 ± 0.56</td>
<td>1.75 ± 0.57</td>
<td>0.24</td>
</tr>
<tr>
<td>Atrophy</td>
<td>1.15 ± 0.50</td>
<td>0.81 ± 0.54</td>
<td>0.03</td>
</tr>
<tr>
<td>Intestinal metaplasia</td>
<td>0.13 ± 0.53</td>
<td>0.00 ± 0.00</td>
<td>0.31</td>
</tr>
<tr>
<td>Bacterial density</td>
<td>1.79 ± 0.68</td>
<td>1.63 ± 0.80</td>
<td>0.48</td>
</tr>
<tr>
<td><strong>Corpus</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Activity</td>
<td>1.06 ± 0.63</td>
<td>0.81 ± 0.65</td>
<td>0.17</td>
</tr>
<tr>
<td>Inflammation</td>
<td>1.31 ± 0.55</td>
<td>0.94 ± 0.57</td>
<td>0.03</td>
</tr>
<tr>
<td>Atrophy</td>
<td>0.42 ± 0.49</td>
<td>0.06 ± 0.25</td>
<td>0.01</td>
</tr>
<tr>
<td>Intestinal metaplasia</td>
<td>0.06 ± 0.43</td>
<td>0.00 ± 0.00</td>
<td>0.56</td>
</tr>
<tr>
<td>Bacterial density</td>
<td>1.81 ± 0.67</td>
<td>1.38 ± 0.71</td>
<td>0.03</td>
</tr>
<tr>
<td>OLGA score</td>
<td>1.15 ± 0.50</td>
<td>0.81 ± 0.54</td>
<td>0.03</td>
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</table>
are cagA positive (Matsuda et al., 2011; Suzuki et al., 2012; Yamaoka et al., 1999b). In Western countries, individuals infected with cagA-positive H. pylori strains reportedly have a higher risk of peptic ulcer or gastric cancer than those infected with cagA-negative strains (van Doorn et al., 1998; Yamaoka et al., 2002). Furthermore, CagA can be divided into two types (East-Asian and Western) according to the repeat sequences of the 3’ region of cagA (Yamaoka, 2010). The repeat regions contain the Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs. Sequences have been annotated according to segments (20–50 aa) flanking the EPIYA motifs (i.e., segments EPIYA-A, -B, -C and -D) (Yamaoka, 2010). Compared with individuals with Western-type CagA strains containing EPIYA-C segments, those infected with East-Asian-type CagA strains containing EPIYA-D segments reportedly have an increased risk of peptic ulcer or gastric cancer (Matsunari et al., 2012; Vilaichone et al., 2004). We determined the prevalence of cagA to be 75.0% in the Dominican Republic, which is similar to that of neighboring countries. The cagA-positive rate is reportedly 73.2% in Cuban strains (Torres et al., 2009). Furthermore, all of the 39 strains sequenced successfully were Western-type CagA; in particular, 84.6% of CagA strains showed the ABC pattern. The prevalence of strains with multiple C segments was only 7.7% and all of them were ABCC, but not ABCCC. The percentage of strains with ABCC was lower than that in Cuban strains (20%) (Torres et al., 2012). In Western countries, the incidence of gastric cancer in patients infected with strains carrying multiple EPIYA-C repeats is higher than the incidence in those infected with strains with a single repeat (Argent et al., 2004; Azuma et al., 2002; Xia et al., 2009; Yamaoka et al., 1998a, 1999a). For example, 31% of H. pylori strains had multiple EPIYA-C repeats in Colombia, where the incidence of gastric cancer is relatively high (17.4:100 000 year−1) (Yamaoka et al., 1999a). Western-type CagA and the lower percentage of multiple EPIYA-C repeats in the Dominican Republic may account for the lower incidence of gastric cancer in the Dominican Republic than in East-Asian countries. However, we found that cagA-positive patients had significantly greater atrophy in the antrum and corpus than cagA-negative patients. Inflammation score in the corpus was also significantly higher in these patients. Interestingly, none of 16 cagA-negative patients had intestinal metaplasia in the antrum or corpus. These results suggest that patients infected with cagA-positive H. pylori strains can be a high-risk population, even in the Dominican Republic.

Many studies in Western countries have shown that individuals infected with vacA s1 or m1 H. pylori strains have a higher risk of peptic ulcer or gastric cancer than those infected with s2 or m2 strains (Atherton et al., 1995; Cover & Blaser, 1992; Sugimoto & Yamaoka, 2009; Sugimoto et al., 2009). The vacA s1m1 genotype was predominant in the Dominican Republic, as in Jamaica and Cuba (Hisada et al., 2001; Torres et al., 2009). On the contrary, 21.9% of subjects had the vacA s2m2 genotype—a prevalence higher than that in Japan, where most cases are vacA s1m1 (Yamaoka et al., 1998b). Furthermore, vacA s2m1 or vacA s2m2 genotypes were found even in cagA-positive strains and this is consistent with findings in Cuban strains (Torres et al., 2009). Less virulent types of vacA may also be related to the lower incidence of gastric cancer in Caribbean countries including the Dominican Republic.

However, our study had several limitations. The prevalence of H. pylori infection in this study was determined based on a combination of four analyses. Although several clinical tests have been developed to diagnose H. pylori infection, no ‘gold standard’ has been established. In this study, histological examination showed the highest positive rate. The histological diagnosis of H. pylori infection is very much dependent on the expertise of the pathologists. Rapid urease tests, such as CLOtest, can be useful for rapid diagnosis. However, the accuracy of this test can be affected by the bacterial load (Megraud & Lehours, 2007) and a reportedly low sensitivity (Megraud, 1997). The results of cultures from biopsy specimens are dependent on transport conditions and careful handling in the laboratory (Megraud & Lehours, 2007). Other global tests, such as the urea breath test or the stool H. pylori antigen test, should be used in future studies.

In conclusion, we found that the prevalence of H. pylori infection in the Dominican Republic was high despite the low incidence of gastric cancer. Lower virulence of H. pylori and mild gastritis in the Dominican Republic might contribute to the low incidence of gastric cancer. However, the presence of H. pylori was related to severe clinical outcomes and more severe gastritis. In addition, the presence of cagA was related to more severe gastritis. Therefore, eradication therapy for H. pylori would likely contribute to decreasing H. pylori-related diseases such as peptic ulcer and gastric cancer in the Dominican Republic.

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