TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2015) 45: 940-946
© TÜBİTAK
doi:10.3906/sag-1409-77

Prevalence of *Helicobacter pylori cagA, babA2,* and *dupA* genotypes and correlation with clinical outcome in Malaysian patients with dyspepsia

Hussein Ali OSMAN[1], Habsah HASAN[1], Rapeah SUPPIAN[2], Syed HASSAN[3], Dzulkarnaen Zakaria ANDEE[3], Noorizan ABDUL MAJID[4], Bin-Alwi ZILFALIL[4,*]

[1]Department of Medical Microbiology and Parasitology, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia

[2]Biomedicine Program, School of Health Sciences, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia

[3]Department of Surgery, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia

[4]Department of Pediatrics, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia

Received: 18.09.2014 • **Accepted/Published Online:** 20.12.2014 • **Printed:** 30.07.2015

Background/aim: The severity of disease outcome in dyspepsia has been attributed to *Helicobacter pylori* virulence genes. The aim of this study was to determine the distribution of *H. pylori* virulence genes (*cagA*, *babA2*, and *dupA*) and to determine whether or not there arises a significant correlation with clinical dyspepsia outcomes.

Materials and methods: *H. pylori* genotypes *cagA*, *babA2*, and *dupA* were identified by polymerase chain reactions from gastric biopsy samples in 105 *H. pylori*-positive patients.

Results: The positive rates for *cagA*, *babA2*, and *dupA* genes in *H. pylori* dyspeptic patients were 69.5%, 41.0%, and 22.9%, respectively. *cagA* was more prevalent in Indians (39.7%), *babA2* was more prevalent in Malays (39.5%), and *dupA* detection occurred more frequently in both Indians and Malays and at the same rate (37.5%). The Chinese inhabitants had the lowest prevalence of the three genes. Nonulcer disease patients had a significantly higher distribution of *cagA* (76.7%), *babA2* (74.4%), and *dupA* (75.0%). There was no apparent association between these virulence genes and the clinical outcomes.

Conclusion: The lower prevalence of these genes and variations among different ethnicities implies that the strains are geographically and ethnically dependent. None of the virulence genes were knowingly beneficial in predicting the clinical outcome of *H. pylori* infection in our subjects.

Key words: *Helicobacter pylori*, *cagA*, *babA2*, *dupA*, ethnicity, virulence genes

1. Introduction

Helicobacter pylori affects more than half of the world's population and over 70% of those inflicted reside in developing countries (1). *H. pylori* colonizes the gastric mucosa, causing chronic gastritis, peptic ulcers, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue lymphoma (2,3). The clinical outcome linked to these diseases has been associated with host genetic factors, environmental factors, and pathogen virulence factors (4). A number of proteins, including vacA, cagA, babA, dupA, SabA, and iceA, have been inferred to play a vital role in the virulence of *H. pylori* by increasing the severity of the disease outcome (5–8).

The cytotoxin-associated gene (*cagA*) is most commonly associated with cytotoxin production and the induction of interleukin 8 (IL-8) by gastric epithelial cells (9). The cag pathogenicity island (PAI), in which the *cagA* gene is localized at one end, is involved in the induction of gastric IL-8 production, though most reports have demonstrated that the cagA protein is not involved in IL-8 induction (10,11). However, one study has verified that *cagA* participates in IL-8 induction in a strain-dependent and time-dependent manner (12). *cagA* is deemed to be one of the most imperative virulence factors in the pathogenesis of *H. pylori*. *cagA* belongs to a cag PAI that codes a type IV secretion system and this secretion system is responsible for the translocation of *cagA* into host cells (13). In Western countries, *cagA*-positive strains are reported to be linked with severe clinical outcomes, but in East Asian countries, it remains abstruse when trying to find this link because almost all *H. pylori* strains possess *cagA* (14).

* Correspondence: zilfalil2@hotmail.com

babA is a blood-group antigen-binding adhesin encoded by the *babA2* gene, which has been shown to bind *H. pylori* to human Lewis b blood-group (Leb) antigens on gastric epithelial cells (15). Although three *bab* alleles have been identified (*babA1*, *babA2*, and *babB*), only the *babA2* gene product is functional for Leb binding activity (16). Some studies discovered a significant relation between *babA2* positive genotypes and the occurrence of peptic ulcer diseases (12), while others failed to find these relationships (17,18).

The duodenal ulcer (DU)-promoting gene (*dupA*) was initially described by Lu et al. in a study examining 14 *vir* gene homologs and their association with gastroduodenal disease, and especially with DU; hence, the gene was named *dupA* (6). *dupA* has been linked to an increased risk of DU and protection against gastric atrophy, intestinal metaplasia, and gastric cancer in Japan and Korea (6). Although some researchers have supported Lu et al.'s theory, others have found no such association. A study conducted within the Iraqi population reported that *dupA* is associated with peptic ulcers (19). In comparison, Argent et al. did not discover any correlation between *dupA* and DU in populations from Belgium, South Africa, China, and the United States (20).

The Malaysian population is divided into three ethnic groups (Malay, Chinese, and Indian) and these groups reflect differences in *H. pylori* infection. There are variations regarding the association between *H. pylori* virulence markers and *H. pylori*-associated diseases from one geographic area to another. Therefore, the aim of the present study was to assess the distribution of *cagA*, *babA2*, and *dupA* in *H. pylori* strains from Malaysia's multiethnic population and to determine its association with clinical outcomes.

2. Materials and methods

This was a prospective study conducted on 226 patients who underwent routine endoscopies from July 2012 to January 2014 in the endoscopy units of Hospital University Sains Malaysia and Hospital Kuala Lumpur. Patients were excluded from the study if they had received treatment with antibiotics, proton pump inhibitors, H2 receptor antagonists, or bismuth compounds within the 4 weeks prior to the study. After the endoscopic examination, the gastric biopsy specimens from the antrum were examined for the presence of *H. pylori* by rapid urease tests, culture, and histology.

This study was approved by the Human Research Ethics Committee, University Sains Malaysia, Kubang Kerian, Kelantan, Malaysia, and the National Medical Research Registry. Written informed consent was obtained from each patient prior to enrollment in the study.

2.1. Rapid urease test

Gastric antral biopsies were collected for the rapid urease test (RUT). The diagnosis of infection was based on the RUT, culture, and histology. All 105 biopsy samples tested positive by RUT. Culture was performed on 81 samples, out of which 33 samples tested positive for *H. pylori*. Out of 30 samples diagnosed by histology, only 20 samples proved positive for *H. pylori*. RUT was performed with a solution of 1 mL of distilled water, one drop 1% of phenol red, and 100 mg of urea. One antral biopsy sample was placed in the solution immediately after endoscopy and maintained at room temperature. The test was considered positive when the color changed from yellow to red within 24 h (21).

2.2. Transport of samples

Biopsy samples for polymerase chain reaction (PCR) were placed in 500 µL of *Brucella* broth with 20% (v/v) glycerol and kept at –80 °C until processing (22).

2.3. Culture and identification of *H. pylori*

Gastric biopsy specimens were inoculated onto Columbia agar base (Oxoid, UK) supplemented with 7% laked horse blood and *H. pylori* Dent's selective (containing 5.0 mg/mL vancomycin, 2.5 mg/mL trimethoprim, 2.5 mg/mL cefsulodin, and 2.5 mg/mL amphotericin B), and the plates were incubated for 5–7 days at 37 °C under microaerophilic conditions. Organisms were identified as *H. pylori* by Gram stain and oxidase, catalase, and urease tests.

2.4. DNA extraction

Genomic DNA was extracted from a gastric biopsy using the QIAamp DNA tissue extraction kit (QIAGEN, Germany) according to the manufacturer's instructions and the DNA was stored at –20 °C until analysis.

2.5. PCR amplifications and conditions

PCR amplifications of *cagA*, *babA2*, and *dupA* were carried out with the use of the primers listed in Table 1 (23–26). The PCR reaction mixtures were prepared using the TopTaq Master Mix Kit (QIAGEN) in a final volume of 25 µL containing 1.25 U of TopTaq DNA polymerase, 1X PCR buffer, 1.5 mM $MgCl_2$, 200 µM of each dNTP, 0.2 µM of each primer, 10 µL of molecular grade water, and 2.5 µL of DNA. The mixtures were placed in a PCR thermocycler (Eppendorf, Germany).

The PCR conditions for *cagA* included an initial denaturation of target DNA at 94 °C for 1 min, followed by 35 cycles of denaturation at 94 °C for 1 min, primer annealing at 58 °C for 1 min, and extension at 72 °C for 1 min, with final extension at 72 °C for 15 min. As for the *babA2* and *dupA* genes, the PCR conditions were: 35 cycles of denaturation at 94 °C for 45 s, primer annealing at 52 °C for 45 s, and extension at 72 °C for 45 s. PCR products were run on 1.5% agarose gels containing red gel in the TBE buffer according to the manufacturer's instructions.

Table 1. Primers used for PCR amplification of *cagA*, *babA2*, and *dupA* genes.

Primer	Primer sequence (5′-3′)	Size base pairs	References
cagA-D008	GGTCAAAATGCGGTCATGG	297	(23,24)
cagA-R008	TTAGAATAATCAACAAACATCACGCCAT		
babA2	CCAAACGAAACAAAAAGCGT	271	(25)
	GCTTGTGTAAAAGCCGTCGT		
*dupA*1	CGTGATCAATATGGATGCTT	197	(26)
*dupA*2	TCTTTCTAGCTTGAGCGA		

2.6. Data analyses

Stata Version 11 (StataCorp, USA) was used for analysis. The chi-square test or Fisher's exact test was applied in order to analyze variances in *H. pylori* virulence genes among gastric ulcer (GU), DU, and gastritis. The statistical significance was set at $P < 0.05$.

3. Results

3.1. Patients and *H. pylori*

Out of 226 patients, 105 (46.5%) were confirmed to be infected with *H. pylori* by RUT. The infected patients (57 males and 48 females) ranged from between 26 to 86 years old (mean age: 54.48 ± 12.94 years). Based on the endoscopic findings, 77 patients were diagnosed with nonulcer dyspepsia (NUD) or gastritis, 9 had GUs, 5 had DUs, and 13 were normal.

3.2. Frequency of *cagA, babA2,* and *dupA*

cagA was detected in 73 (69.5%) of the biopsy samples. The distribution of *cagA* among the three groups (Indian, Malay and Chinese) was 29 (39.7%), 26 (35.6%), and 18 (24.7%), respectively (Table 2). The Indian population exhibited the highest distribution of *cagA* compared to the others.

The *babA2* gene was observed in 43 (41.0%) patients derived from biopsy samples. The distribution of the *babA2* gene among Indian, Malay, and Chinese populations was 14 (32.6%), 17 (39.5%), and 12 (27.9%), respectively (Table 2).

The *dupA* gene was also found in 24 (22.9%) biopsy samples. The distribution of the *dupA* gene among Indian, Malay, and Chinese inhabitants was 9 (37.5%), 9 (37.5%), and 6 (25.0%), respectively (Table 2).

3.3. *cagA, babA2,* and *dupA* genes and clinical outcome

The prevalence of *cagA* was higher in the NUD group (76.7%) than in the GU (11.0%), DU (4.1%), and normal groups (8.2%). Similarly, *babA2* was most prevalent in NUD (77.1%) patients. *dupA* was more frequent in the NUD group (75.0%) than in the others (Table 3). The endoscopic findings (NUD, DU, GU, and normal group) were higher in male patients (54.3%) than in females (45.7%). Overall, there was no significant difference between *cagA*, *babA2*, and *dupA* genes and clinical outcomes (Table 3).

A combination of *cagA*, *babA2*, and *dupA* was detected in 15 biopsy samples and a combination of *cagA* and *babA2* was noted in 38 biopsy samples. Twenty-one patients had a combination of both *cagA* and *dupA*. A total of 16 biopsy samples tested positive for *babA2* and *dupA*, as indicated in Table 4. There was no significant variance observed between the combinations and clinical outcomes.

4. Discussion

The clinical development of *H. pylori* infection depends on a combination of many factors pertaining to both the host and the bacteria. Among the bacterial factors, studies have revealed that certain *H. pylori* genotypes cause more severe pathologies (27).

Table 2. Distribution of *cagA*, *babA2*, and *dupA* gene by ethnicity.

Ethnic group (n)	*cagA*		*babA2*		*dupA*	
	Positive	Negative	Positive	Negative	Positive	Negative
Indian (37)	29 (39.7)	8 (25.0)	14 (32.6)	23 (37.1)	9 (37.5)	28 (34.6)
Malay (42)	26 (35.6)	16 (50.0)	17 (39.5)	25 (40.3)	9 (37.5)	33 (40.7)
Chinese (26)	18 (24.7)	8 (25)	12 (27.9)	14 (22.6)	6 (25.0)	20 (24.7)

Table 3. Distribution of *cagA*, *babA2*, and *dupA* and clinical outcome in *H. pylori*-infected patients.

Sex	NUD n (%)	GU n (%)	DU n (%)	Normal n (%)	*P-value
Male	44 (56.4)	4 (44.4)	4 (80.0)	5 (38.5)	0.371
Female	34 (43.6)	5 (55.6)	1 (20.0)	8 (61.5)	
Virulence genes					
cagA+	56 (76.7)	8 (11.0)	3 (4.1)	6 (8.2)	0.146
cagA -	22 (68.8)	1 (3.1)	2 (6.2)	7 (21.9)	
babA2+	32 (74.4)	5 (11.6)	3 (7.0)	3 (7.0)	0.290
babA2-	46 (74.2)	4 (6.5)	2 (3.2)	10 (16.1)	
dupA+	18 (75.0)	1 (4.2)	2 (8.3)	3 (12.5)	0.700
dupA-	60 (74.1)	8 (9.9)	3 (3.7)	10 (12.3)	

NUD = Nonulcer disease (gastritis); GU = gastric ulcer; DU = duodenal ulcer.
*Fisher's exact test was applied.

Table 4. Combined *cagA*, *babA2*, and *dupA* genotypes and clinical outcome.

Virulence genes	NUD n (%)	GU n (%)	DU n (%)	Normal n (%)	*P-value
cagA+/*babA2*+/*dupA*+	11 (32.4	1 (20.0)	1 (33.3	2 (66.7)	0.678
cagA-/*babA2*-/*dupA*-	23 (67.6)	4 (80.0)	2 (66.7)	1 (33.3)	
cagA+/*babA2*+	28 (82.4)	5 (100)	2 (66.7)	3 (100)	0.549
cagA-/*babA2*-	6 (17.6)	0 (0.0)	1 (33.3)	0 (0.0)	
cagA+/*dupA*+	16 (47.1)	1 (20.0)	2 (66.7)	2 (66.7)	0.565
cagA-/*dupA*-	18 (52.9)	4 (80.0)	1 (33.3)	1 (33.3)	
babA2+/*dupA*+	12 (35.3)	1 (20.0)	1 (33.3)	2 (66.7)	0.654
babA2-/*dupA*-	22 (64.7)	4 (80.0)	2 (66.7)	1 (33.3)	

NUD = Nonulcer disease (gastritis); GU = gastric ulcer; DU = duodenal ulcer.
*Fisher's exact test was applied.

In this study, we determined the frequency of *cagA*, *babA2*, and *dupA* in dyspeptic patients. The prevalence of *cagA* differs in every part of the world. The prevalence of *cagA* is lower in Western countries (28,29) when compared to East Asian countries, where *cagA* is present in more than 90% of cases irrespective of clinical presentation (30).

The prevalence of *cagA* in this study was 69.5%; this is slightly lower than reports from East Asian countries. Studies conducted locally have reported differing percentages. According to Ramelah et al., the prevalence of *cagA* was 94% (31), while Amjad et al. reported 43% (32). This divergence within the same country may well be due to differences in sample size, primer sets, or the variety of strains within the same country. In addition, the results of this study did not elaborate on conclusive evidence linking *cagA* with NUD patients; our results are in agreement with supplementary studies in Asian countries (31,33) that failed to find any association. However, various studies have reported that *cagA* was statistically concomitant with peptic ulcers (34,35).

babA2 attaches *H. pylori* to these cells, enabling delivery of vacA and *cagA* toxins near the gastric epithelium and therefore increasing gastric tissue damage (15). The prevalence of the *babA2* genotype in our study was 41.0%.

Our results are consistent with a study conducted in China, which reported a prevalence rate of 38.9% in dyspeptic patients (36), but slightly lower than studies from Turkey (53.8%) (34). Oliveira et al. discovered that *babA2* was more frequently found in patients with DU and gastric cancer (35). The current study did not include a gastric cancer case and DU accounts only for 4.8% of the studied population, so this might have contributed to the low prevalence of *babA2* in our study. Gerhad et al. discovered that *babA2* was associated with peptic ulcer disease in Western populations (15). Our study failed to find any substantial associations between *babA2* and the clinical outcome. This is in agreement with a previous conducted study (18).

During the present study, 24 (22.9%) *dupA*-positive *H. pylori* strains were observed in the patients' biopsy samples. Our data are in line with a study of Japanese patients (21.3%) (6) and slightly lower than the findings of a study conducted in China (35.3%) (37). The only other study performed in Malaysia also unearthed a comparable prevalence of 21.3% (38). *dupA* has been linked to an increased risk of DU and a decreased risk of gastric cancer (6). In contrast, Lu et al. (6) did not observe an association between *dupA* and DU in East Asia. These differences in results might be due to strain variations moving from one region to another. Furthermore, the DU patients used in our study account for only 4.8% of the study group, which prevents us from drawing a definitive conclusion.

Combinations of two or three of the virulence genes were not noticeably diverse among the NUD, GU, DU, and normal groups in our study, although a study conducted in Cuba reported a significant association between a combination of *cagA* and *babA2* genotypes (39).

In our study, NUD patients tended to have a higher distribution of *cagA* (76.7%), *babA2* (74.4%), and *dupA* (75.0%) compared to peptic ulcer disease. Our result is in agreement with a study from Iran, which found an obviously much higher prevalence of the *cagA* gene (73%) in NUD patients (40). Others have found a higher presence of *cagA* in peptic ulcer disease patients than in NUD (31,41). This dissimilarity might be due to an imbalance in NUD and peptic ulcer disease cases.

In conclusion, although there is no association between virulence genotypes and clinical outcomes in our study, the lower prevalence of these genotypes in *H. pylori*-positive patients and variations among different ethnicities indicates that there is strain variation among countries and ethnic groups.

Acknowledgments

This study was supported by Research University Grant Number 1001 / PPSP / 812108. We would like to thank the Islamic Development Bank for giving the first author a scholarship.

References

1. Frenck RW, Clemens J. *Helicobacter* in the developing world. Microbes Infect 2003; 5: 705–713.
2. Ben Mansour K, Fendri C, Zribi M, Masmoudi A, Labbene M, Fillali A, Ben Mami N, Najjar T, Meherzi A, Sfar T et al. Prevalence of *Helicobacter pylori vacA*, *cagA*, *iceA* and *oipA* genotypes in Tunisian patients. Ann Clin Microbiol Antimicrob 2010; 9: 10.
3. Zhao Y, Wang J, Tanaka T, Hosono A, Ando R, Soeripto S, Ediati Triningsih FX, Triono T, Sumoharjo S, Astuti EY et al. Association between HLA-DQ genotypes and haplotypes vs *Helicobacter pylori* infection in an Indonesian population. Asian Pac J Cancer Prev 2012; 13: 1247–1251.
4. Yamaoka Y. Roles of the plasticity regions of *Helicobacter pylori* in gastroduodenal pathogenesis. J Med Microbiol 2008; 57: 545–553.
5. Wu CC, Chou PY, Hu CT, Liu ZC, Lin CY, Tseng YH, Lin NT. Clinical relevance of the *vacA*, *iceA*, *cagA*, and *flaA* genes of *Helicobacter pylori* strains isolated in Eastern Taiwan. J Clin Microbiol 2005; 43: 2913–2915.
6. Lu H, Hsu PI, Graham DY, Yamaoka Y. Duodenal ulcer promoting gene of *Helicobacter pylori*. Gastroenterology 2005; 128: 833–848.
7. Aspholm M, Olfat FO, Norden J, Sonden B, Lundberg C, Sjostrom R, Altraja S, Odenbreit S, Haas R, Wadstrom T et al. SabA is the *H. pylori* hemagglutinin and is polymorphic in binding to sialylated glycans. PLoS Pathog 2006; 2: e110.
8. Atherton JC. The pathogenesis of *Helicobacter pylori*-induced gastro-duodenal diseases. Annu Rev Pathol 2006; 1: 63–96.
9. Jenks PJ, Megraud F, Labigne A. Clinical outcome after infection with *Helicobacter pylori* does not appear to be reliably predicted by the presence of any of the genes of the cage pathogenicity island. Gut 1998; 43: 752–758.
10. Audibert C, Janvier B, Grignon B, Salaün L, Burucoa C, Lecron JC, Fauchère JL. Correlation between IL-8 induction, *cagA* status and *vacA* genotypes in 153 French *Helicobacter pylori* isolates. Res Microbiol 2000; 151: 191–200.
11. Odenbreit S, Kavermann H, Püls J, Haas R. *CagA* tyrosine phosphorylation and interleukin-8 induction by *Helicobacter pylori* are independent from alpAB, HopZ and bab group outer membrane proteins. Int J Med Microbiol 2002; 292: 257–266.
12. Brandt S, Kwok T, Hartig R, König W, Backert S. NF-κB activation and potentiation of proinflammatory responses by the *Helicobacter pylori CagA* protein. P Natl Acad Sci USA. 2005; 102: 9300–9305.

13. Naito M, Yamazaki T, Tsutsumi R, Higashi H, Onoe K, Yamazaki S, Azuma T, Hatakeyama M. Influence of EPIYA-repeat polymorphism on the phosphorylation-dependent biological activity of *Helicobacter pylori* CagA. Gastroenterology 2006; 130: 1181–1190.

14. Yamaoka Y. Mechanisms of disease: *Helicobacter pylori* virulence factors. Nat Rev Gastroenterol Hepatol 2010; 7: 629–641.

15. Gerhard M, Lehn N, Neumayer N, Boren T, Rad R, Schepp W, Miehlke S, Classen M, Prinz C. Clinical relevance of the *Helicobacter pylori* gene for blood-group antigen-binding adhesin. P Natl Acad Sci USA 1999; 96: 12778–12783.

16. Pride DT, Meinersmann RJ, Blaser MJ. Allelic variation within *Helicobacter pylori* babA and babB. Infect Immun 2001; 69: 1160–1171.

17. Talebi Bezmin Abadi A, Taghvaei T, Mohabbati Mobarez A, Vaira G, Vaira D. High correlation of babA 2-positive strains of *Helicobacter pylori* with the presence of gastric cancer. Intern Emerg Med 2013; 8: 497–501.

18. Abdollahi H, Shokoohi M, Savari M. The prevalence of *Helicobacter pylori babA2*, *iceA1* and *iceA2* genes and their association with clinical outcomes in patients with chronic gastritis, ulcerative diseases and non-ulcer dyspepsia in south east of Iran. Jundishapur J Microbiol 2013; 6: e4739.

19. Hussein NR, Mohammadi M, Talebkhan Y, Doraghi M, Letley DP, Muhammad MK, Argent RH, Atherton JC. Differences in virulence markers between *Helicobacter pylori* strains from Iraq and those from Iran: potential importance of regional differences in *H. pylori*-associated disease. J Clin Microbiol 2008; 46: 1774–1779.

20. Argent RH, Burette A, Miendje Deyi VY, Atherton JC. The presence of *dupA* in *Helicobacter pylori* is not significantly associated with duodenal ulceration in Belgium, South Africa, China, or North America. Clin Infect Dis 2007; 45: 1204–1206.

21. Pourakbari B, Mirsalehian A, Maleknejad P, Mamishi S, Azhdarkosh H, Daryani NE, Najafi M, Kazemi B, Paknejad M, Mahmoudi S et al. Evaluation of a new antigen for diagnosis of *Helicobacter pylori* infection in stool of adult and children. Helicobacter 2011; 16: 42–46.

22. Arevalo-Galvis A, Trespalacios-Rangell AA, Otero W, Mercado-Reyes MM, Poutou-Pinales RA. Prevalence of *cagA*, *vacA*, *babA2* and *iceA* genes in *H. pylori* strains isolated from Colombian patients with functional dyspepsia. Pol J Microbiol 2012; 61: 33–40.

23. Domingo D, Alarcon T, Prieto N, Sanchez I, Lopez-Brea M. *cagA* and *vacA* status of Spanish *Helicobacter pylori* clinical isolates. J Clin Microbiol 1999; 37: 2113–2114.

24. Sillakivi T, Aro H, Ustav M, Peetsalu M, Peetsalu A, Mikelsaar M. Diversity of *Helicobacter pylori* genotypes among Estonian and Russian patients with perforated peptic ulcer, living in Southern Estonia. FEMS Microbiol Lett 2001; 195: 29–33.

25. Sheu BS, Sheu SM, Yang HB, Huang AH, Wu JJ. Host gastric Lewis expression determines the bacterial density of *Helicobacter pylori* in *babA2* genopositive infection. Gut 2003; 52: 927–932.

26. Gomes LI, Rocha GA, Rocha AM, Soares TF, Oliveira CA, Bittencourt PF, Queiroz DM. Lack of association between *Helicobacter pylori* infection with *dupA*-positive strains and gastroduodenal diseases in Brazilian patients. Int J Med Microbiol 2008; 298: 223–230.

27. Hocker M, Hohenberger P. *Helicobacter pylori* virulence factors—one part of a big picture. Lancet 2003; 362: 1231–1233.

28. Ribeiro ML, Godoy AP, Benvengo YH, Mendonca S, Pedrazzoli J. Clinical relevance of the *cagA*, *vacA* and *iceA* genotypes of *Helicobacter pylori* in Brazilian clinical isolates. FEMS Immunol Med Microbiol 2003; 36: 181–185.

29. Podzorski RP, Podzorski DS, Wuerth A, Tolia V. Analysis of the *vacA*, *cagA*, *cagE*, *iceA*, and *babA2* genes in *Helicobacter pylori* from sixty-one pediatric patients from the Midwestern United States. Diagn Microbiol Infect Dis 2003; 46: 83–88.

30. Yamaoka Y, Orito E, Mizokami M, Gutierrez O, Saitou N, Kodama T, Osato MS, Kim JG, Ramirez FC, Mahachai V et al. *Helicobacter pylori* in North and South America before Columbus. FEBS Lett 2002; 517: 180–184.

31. Ramelah M, Aminuddin A, Alfizah H, Isa MR, Jasmi AY, Tan HJ, Rahman AJ, Rizal AM, Mazlam MZ. *cagA* gene variants in Malaysian *Helicobacter pylori* strains isolated from patients of different ethnic groups. FEMS Immunol Med Microbiol 2002; 44: 239–242.

32. Amjad N, Osman HA, Razak NA, Kassian J, Din J, bin Abdullah N. Clinical significance of *Helicobacter pylori cagA* and *iceA* genotype status. World J Gastroenterol 2010; 16: 4443–4447.

33. Zheng PY, Hua J, Yeoh KG, Ho B. Association of peptic ulcer with increased expression of Lewis antigens but not *cagA*, *iceA*, and *vacA* in *Helicobacter pylori* isolates in an Asian population. Gut 2000; 47: 18–22.

34. Erzin Y, Koksal V, Altun S, Dobrucali A, Aslan M, Erdamar S, Dirican A, Kocazeybek B. Prevalence of *Helicobacter pylori vacA*, *cagA*, *cagE*, *iceA*, *babA2* genotypes and correlation with clinical outcome in Turkish patients with dyspepsia. Helicobacter 2006; 11: 574–580.

35. Oliveira AG, Santos A, Guerra JB, Rocha GA, Rocha AM, Oliveira CA, Cabral MM, Nogueira AM, Queiroz DM. *babA2*- and *cagA*-positive *Helicobacter pylori* strains are associated with duodenal ulcer and gastric carcinoma in Brazil. J Clin Microbiol 2003; 41: 3964–3966.

36. Zheng PY, Tang FA, Qi YM, Li J. Association of peptic ulcer with increased expression of Lewis antigens, but not vacuolating cytotoxin activity or *babA2* gene status, in *Helicobacter pylori* strains from China. Chin J Dig Dis 2006; 7: 61–65.

37. Zhang Z, Zheng Q, Chen X, Xiao S, Liu W, Lu H. The *Helicobacter pylori* duodenal ulcer promoting gene, *dupA* in China. BMC Gastroenterol 2008; 8: 49.

38. Schmidt HM, Andres S, Kaakoush NO, Engstrand L, Eriksson L, Goh KL, Fock KM, Hilmi I, Dhamodaran S, Forman D et al. The prevalence of the duodenal ulcer promoting gene (*dupA*) in *Helicobacter pylori* isolates varies by ethnic group and is not universally associated with disease development: a case-control study. Gut Pathog 2009; 1: 5.

39. Torres LE, Melián K, Moreno A, Alonso J, Sabatier CA, Hernández M, Bermúdez L, Rodríguez BL. Prevalence of *vacA*, *cagA* and *babA2* genes in Cuban *Helicobacter pylori* isolates. World J Gastroenterol 2009; 15: 204–210.

40. Dabiri H, Maleknejad P, Yamaoka Y, Feizabadi MM, Jafari F, Rezadehbashi M, Nakhjavani FA, Mirsalehian A, Zali MR. Distribution of *Helicobacter pylori cagA*, *cagE*, *oipA* and *vacA* in different major ethnic groups in Tehran, Iran. J Gastroenterol Hepatol 2009; 24: 1380–1386.

41. Alaoui Boukhris S, Benajah DA, El Rhazi K, Ibrahimi SA, Nejjari C, Amarti A, Mahmoud M, El Abkari M, Souleimani A, Bennani B et al. Prevalence and distribution of *Helicobacter pylori cagA* and *vacA* genotypes in the Moroccan population with gastric disease. Eur J Clin Microbiol Infect Dis 2012; 31: 1775–1781.