Microbiology Discovery
ISSN 2052-6180

HOAJ
Herbert Open Access Journals

Special Section | Microbial Pathogenesis | Research Open Access

Prevalence of *H. pylori* strains harbouring *cagA* and *iceA* virulence genes in saudi patients with gastritis and peptic ulcer disease

Roqayah H. Kadi[1], Eman M. Halawani[1] and Hayam S. Abdelkader[2*]

*Correspondence: hayamabdelkader68@yahoo.com

[1]Biology Department, Faculty of Science, Taif University, Kingdom of Saudi Arabia.
[2]Biotechnology Department, Faculty of Science, Taif University, Kingdom of Saudi Arabia.

Abstract

Aim: The study is aimed to detect the presence of *cagA*, *iceA*1, and *iceA*2 virulence genes in *H. pylori* from gastric biopsies, and to deduce the correlation between these genotypes and the two clinical outcomes peptic ulcer disease (PUD), and gastritis.

Materials and methods: Thirty three Saudi patients 15 males and 18 females, 20 to 90 years were assigned into two groups PUD and gastritis. Genomic DNAs were extracted from biopsy specimens and used to detect the presence of *cagA*, and *iceA* genes by PCR typing system. Fisher's and Phi coefficient association tests were used for statistical analysis.

Results: Genotyping show that both *cagA* and *iceA* genes were amplified from 27 specimens (81.7%). The prevalence of *cagA*+ and *cagA*- genotypes or *iceA*+ and *iceA*- genotypes did not differ significantly between males and females (p=0.070). Within the PUD and gastritis groups, the percentages of specimens positive for *cagA* gene were 76.9 % and 85 %, while those positive for *iceA*+ were 92.3% and 75% respectively. All *cagA*+/*iceA*+ combined genotypes was statically correlated with peptic ulcer (77%). This correlation was not observed within *H. pylori* specimens typed from gastritis. Patients with either PUD or gastritis were most likely infected by several strains of *H. pylori*.

Conclusion: Different strains of *H. pylori* have virulent genotypes evidenced by PCR-based genotyping from biopsy specimens at a reasonable cost and time. These virulence strains spread at Taif province, may result in sever clinical outcomes such as ulcers which may be developed to cancer, the situation which necessitates further studies.

Keywords: *H. pylori*, *cagA* genotype, *iceA*+ genotype, peptic ulcer, gastritis, PCR-based genotyping, gastric biopsies

Introduction

Helicobacter pylori colonizing the human stomach acquired by contaminated water or food or poorly disinfected endoscopes. Lifetime persistence of this organism within the host could result in a number of gastroduodenal diseases ranging from mild gastritis, atrophic gastritis, and peptic ulcer disease to malignant diseases such as gastric adenocarcinoma and Mucosa-Associated-Lymphoid-Tissue (MALT) Lymphoma [1,2].

Although a chronic active gastritis will be developed by most of infected patients, the majorities of infections are asymptomatic [1-3]. Found that, 15–20% of infected patients will develop gastric or duodenal ulcer disease and less than 1% will develop gastric adenocarcinoma. Due to poor correlation between symptoms and disease, many of gastric cancer cases are detected lately when the disease is rooted and become incurable. Direct PCR methods performed on *H. pylori* DNA isolated from biopsy specimens have been evaluated previously [4-7].

In developing countries, *H. pylori* infection is particularly high (up to 80%) [9-11]. The prevalence of *H. pylori* infection in Jordan and Bahrain was 77.5% and 79%, respectively [12,13]. In Kuwait and Egypt, *H. pylori* were present in 84% and 86% of the biopsy samples, respectively [14,15] while the rate of infections reached 87% in the Eastern region and 61.6% in Central and Western region in the Kingdom of Saudi Arabia [16,17]. Although some studies have reported an excess of *H. pylori* in one gender versus the other [18,19], found no gender differences in *H. pylori* prevalence overall.

Over the past few years, research on pathogenicity markers has become gradually more important and intense in an attempt to detect bacterial strains associated with each of these diseases. The cytotoxin-associated gene A (*cagA* gene) was the first virulence factor detected in *H. pylori* strains. This gene encodes a protein that is associated with an increase in intensity of gastric inflammation and, consequently, with severe clinical outcomes, inducing an intense inflammatory process, with dense neutrophil infiltration, which can cause serious hurt to the gastric mucosa. The induced by contact with epithelium (*iceA*) gene has two allelic forms, *iceA*1 and *iceA*2 [18,19].

The expression of *iceA*1 was controlled by contact between *H. pylori* and human epithelial cells [20] and the *iceA*1 genotype was associated with enhanced mucosal interleukin (IL)-8 expression and acute antral inflammation [21].

Although *iceA* gene has no correlation with gastric cancer

 doi: 10.7243/2052-6180-2-2

development, there is an inconclusive argument about the role of this gene in gastric pathology. Although [20,22], proved the role of *iceA*1 allele in peptic ulcer, others did not find any role for this allele in gastroduodenal disease [22] while, [23] reported an inverse association between the *iceA*2 allele and peptic ulcer.

Several studies were not able to explain the role of *iceA* and its correlation with clinical outcomes in other populations; therefore the mechanism of how *iceA* induce PUD remains unclear [24,25]. Such contradicted results between the *iceA* genotype and clinical consequences could be explicated by the genetic diversity or differences in the geographic region, which were previously reported for other virulence factors [26]. Moreover, geographic variations in addition to genetic heterogeneity of the host further contribute to the diversity of host responses to particular *H. pylori* strains and genotypes [27].

The objectives of current study are to detect the virulence genes (*cagA*, *iceA*1 and *iceA*2) by polymerase chain reaction (PCR) in gastric biopsy specimens and to find out the possible association between these virulence genotypes and the clinical outcomes.

Subjects and methods

Sample collection

Thirty three biopsy specimens were collected from 15 males and 18 females attending the endoscopy clinic at three hospitals; King Faisal, AL Hada Armed Forces, and King AbdulAziz in Taif province, Kingdome of Saudi Arabia between September 2011 and February 2012. Mean age (± 47) was varying from 20 to 90 years. These patients were scheduled for gastroscopy by their physician based on various symptoms such as abdominal pain, reflux and dyspepsia.

The study was approved by the ethics committee of Taif University and each hospital has obtained an informed consent from each patient prior to performing the study.

The gastric biopsies were transferred immediately into sterile tubes containing 3 ml of saline or Brucella broth labeled with the patient's I.D. and date. Samples were brought directly to the lab for immediate processing. *Campylobacter*-like organism(CLO) test was done on the 33 mucosal specimens. Patients were assigned in the following groups based on the gastroenterologist's diagnosis:

1. PUD group: when there was evidence of erosion or ulceration in the gastric mucosa with exudates and erythema.
2. Gastritis group: when there was evidence of inflammation, edema, punctuate hemorrhage, friability, or nodularity.

The biopsy specimens were fragmented using a sterile pestle and mortar. Further homogenization was done by passing the lysate alternatively 5 times through a 0.9 mm needle (20 gauges) fitted to a syringe. The resulting lysate was divided into aliquots and placed into a microcentrifuge tube for DNA extraction.

Genomic DNA isolation

Genomic DNAs were extracted from thirty three biopsy specimens using the QIAamp DNA mini kit (Qiagen GmbH, Hilden, Germany), as described by the manufacturer. The tissue biopsies were centrifuged at 5000 xg for 10 min and re-suspended in 200 µl of ATL buffer (supplied in the QIAamp DNA Mini Kit) for complete lysis. Finally, the DNAs were eluted in100 µL of elution buffer. DNA purity and quantity was determined using a GeneSys 10UV spectrophotometer (Thermo Scientific, USA).

Genotyping-PCR

Isolated genomic DNAs (gDNAs) were used to detect the presence of *cagA*, and *iceA* by PCR. Primers used for *cagA* gene amplification were as follows: forward primer cagA F1 (5′-GATAACAGCCAAGCTTTTGAGG-3′) and reverse primer cagA B1 (5′-CTGCAAAAGATTGTTTGGCAGA-3′) to amplify 349 bp fragment; cagA F2(5′-AATACACCAACGCCTCCAAG-3′) and cagA B2 (5′-TTGTTGCCGCTTGCTCTC-3′) to amplify 400 bp fragment. The primers used for *iceA*1 amplification were iceA1F(5′- GTGTTTTTAACCAAAGTATC-3′) and iceA1 R (5′-CTATAGCCASTYTCTTTGCA-3′) to amplify 247 bp; and *iceA*2 amplification; iceA2 F (5′-GTTGGGTATATCACAATTTAT-3′) and iceA2 R (5′- TTRCCCTATTTTCTAGTAGGT-3′) to amplify either 229 or 334 bp depending on the number of 105-bp repeated insertions. Each PCR reaction was carried out in a final volume of 25 µl as follows: *cagA*: 13µl of molecular grade water (Qiagen), 1X PCR buffer (Qiagen), 200 µM dNTPs (Qiagen), 0.6 µM primers, 1.5 mM $MgCl_2$ (Qiagen), 0.25 U of Taq DNA polymerase (Qiagen) and 2.5 µl of DNA. *iceA*1and/or *iceA*2: 13µl of molecular grade water (Qiagen), 1X PCR buffer (Qiagen), 200 µM dNTPs (Qiagen), 0.6 µM of each forward and reverse primer, 1.5 mM $MgCl_2$ (Qiagen), 0.25 U of Taq DNA polymerase (Qiagen) and 2.5 µl of DNA. DNA fragments were visualized by UV transillumination(Biometra, GmbH, Germany).

Statistical analysis

Fisher's exact and Phi coefficient association tests were used for analysis of two-by-two and two-by-four- tables of categorical data. All tests were two-tailed, and the significance level was assumed as 0.05.

Results

The distribution of *cagA*+, and *cagA*- genotypes within collected samples is shown in (**Table 1** and **Figure 1**). The PCR-based amplification showed that 27 cases (81.7%) were *cagA*+, while 6 cases (18.8%) were *cagA*-. The percentages of *cagA*+ were 77% (10/13) and 85% (17/20) for PUD and gastritis cases, respectively. The percentage of *cagA*+ genotype within each clinical outcome was significantly higher than that of *cagA*- genotype ($p<0.001$). However, the prevalence of *cagA*+ and *cagA*- genotypes did not differ significantly between the two clinical

doi: 10.7243/2052-6180-2-2

Table 1. Prevalence of *H. pylori* genotypes detected in 33 gastric biopsy specimens enrolled in the current study.

Samples No.	*cagA*	*iceA*1	*iceA*2	gender	diagnosis
A1	+	+	+	M	PUD
A2	-	-	+	F	PUD
A3	+	+	+	M	PUD
A4	-	-	-	M	G
A5	+	+	+	F	PUD
A6	+	+	+	F	G
A7	+	+	+	F	PUD
A8	+	+	+	F	G
A9	+	+	+	M	G
A10	+	+	+	M	G
A11	+	+	+	F	PUD
A12	+	+	+	F	G
A13	+	+	+	F	G
F1	-	+	-	M	G
F2	+	-	-	M	G
F3	-	+	+	F	PUD
F4	+	+	+	F	PUD
F5	+	+	+	M	PUD
F6	+	+	+	F	PUD
F7	+	-	+	M	G
F8	-	-	-	M	PUD
F9	+	+	+	F	G
F10	+	-	+	F	G
F11	+	-	+	F	G
F12	+	+	+	F	PUD
H1	+	-	-	M	G
H2	+	+	+	F	G
H3	-	-	+	M	G
H4	+	-	+	F	G
H5	+	-	-	M	G
H6	+	+	+	M	PUD
H7	+	-	-	F	G
H8	+	-	+	M	G

(*cagA*): cytotoxin-associated gene. (*iceA*1): induced by contact with epithelium allele A1. (*iceA*2): induced by contact with epithelium allele A2. (+): Positive. (-) : Negative. (M): Male. (F):Female. (PUD): Peptic Ulcer Disease.(G): Gastritis.

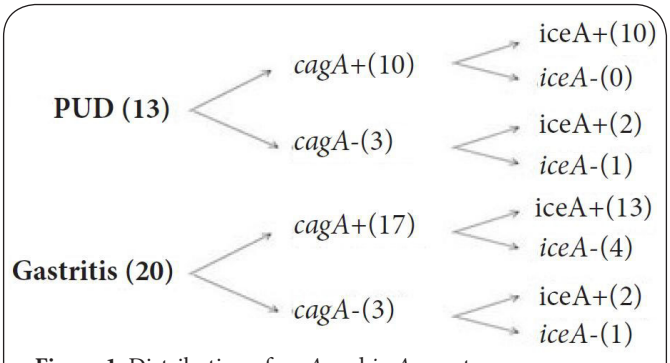

Figure 1. Distribution of *cagA* and *iceA* genotypes among peptic ulcer and gastritis patients. The numbers of patients are shown in parentheses.

outcomes (p=0.658). There was also no association between *cagA*+ and *cagA*- genotypes and gender of patients (p=0.070). Likewise, 27 cases (81.7%) were *iceA*+, while 6 cases (18.8%) were *iceA*- by PCR. The percentage of *iceA*+ genotypes were 92.3% (12/13) and 75% (15/20) for PUD and gastritis cases, respectively. The percentage of *iceA*+ genotype within each clinical outcome was significantly higher than that of *iceA*- genotype. The percentages of *iceA*+ genotype differed significantly between the two clinical outcomes, as *iceA*+ genotype was detected more frequently in PUD patients as compared with gastritis patients. As with *cagA* gene, there was no association between *iceA* genotypes and gender of patients.

In the current study, 93.9% (31/33 cases) of *H. pylori* positive specimens had at least one of the two virulence genes examined in this study, i.e., *cagA* and/or *iceA* (**Figure 1**). This suggests that the majority of *H. pylori* isolates examined in this study had virulence potential as evidenced by PCR-based molecular testing.

The prevalence of the combined *cagA* and *iceA* genotypes among the 13 peptic ulcer and 20 gastritis cases is shown in **Figure 1**. The percentage of *cagA*+/*iceA*+ genotype was significantly high (p<0.001) within peptic ulcer (76.9%) and also within gastritis (65%) cases. However, no association was revealed between the prevalence of the four genotypes (++, +-, -+, --) and the clinical outcome by using 2x4 Fisher's exact test (p=0.498). **Figure 1** shows that all of the *cagA*+ *H. pylori* specimens (n=10) that were typed from peptic ulcer cases were also found to have the *iceA*+ genotype. Of the 17 *cagA*+ *H. pylori* specimens that were typed from gastritis patients, 13 specimens had the *iceA*+ genotype. The *cagA*+ genotype, therefore, could be a predictive marker for the *iceA*+ genotype in *H. pylori* specimens isolated from peptic ulcer patients. This association was not observed within *H. pylori* specimens typed from gastritis cases.

The occurrence of *iceA*1 and *iceA*2 double positive genotypes within the studied samples are shown in **Table 2**. Out of the 33 samples examined 19 (58%) were double positive for *iceA*1 and *iceA*2 genes. These *iceA*1 and *iceA*2 double positives were found in 11 PUD (11/13=85%) and 8 gastritis (8/20=40%) cases. **Table 2** shows also that *cagA*/*iceA*1/*iceA*2 positives occurred in 77% (10/13 cases) of PUD cases and in only 40% (8/20 cases) of gastritis cases. There was a significant association between the occurrence *iceA*1 and *iceA*2 double positives and PUD (p=0.0188). Thus, it appears that infection with multiple strains of *H. pylori* occurs more frequently in patients with PUD, compared to those with gastritis.

Discussion

Our study determined the *cagA* and *iceA* geneotype of *H. pylori* biopsy samples in a group of patients attending the endoscopy clinic at Al-Hada Armed Forces Hospital, King Faisal Hospital, and King Abdul Aziz Hospital.

In this study, PCR was used to characterize *H. pylori* infections

doi: 10.7243/2052-6180-2-2

Table 2. Occurrenceof *iceA*1/*iceA*2 double positives among all cases studied.

PUD and Gastritis cases (n = 33):			
	***cagA*+**	***cagA*-**	**Total**
*iceA*1	0	1 (3%)	1 (3%)
*iceA*2	5 (15%)	2 (6.0%)	7 (21%)
*iceA*1/*iceA*2	18 (54.5%)	1 (3%)	19 (57.5%)
iceA-	4 (12%)	2 (6%)	6 (18%)
Total	27 (81.8%)	6 (18%)	33 (100%)
PUD cases (n = 13):			
	***cagA*+**	***cagA*-**	**Total**
*iceA*1	0	0	0
*iceA*2	0	1 (8%)	1 (8%)
*iceA*1/*iceA*2	10 (77%)	1 (8%)	11 (85%)
iceA-	0	1 (8%)	1 (8%)
Total	10 (77%)	3 (23%)	13 (100%)
Gastritis cases (n = 20):			
	***cagA*+**	***cagA*-**	**Total**
*iceA*1	0	1 (5%)	1 (5%)
*iceA*2	5 (25%)	1 (5%)	6 (30%)
*iceA*1/*iceA*2	8 (40%)	0	8 (40%)
iceA-	4 (20%)	1 (5%)	5 (25%)
Total	17 (85%)	3 (15%)	20 (100%)

in biopsy specimens and to examine the association between genotypes and clinical outcomes. The cytotoxin associated gene A (*cagA* gene) has been proposed as a marker for a genomic pathogenicity island (cag-PAI) of approximately 40 kbp whose presence is associated with more severe clinical outcomes [28,29]. The induced by contact with epithelium gene (*iceA* gene) has recently been discovered [20]. The two main allelic variants of the gene are *iceA*1 and *iceA*2. The expression of *iceA*1 is upregulated on contact between *H. pylori* and human epithelial cells, and may be associated with peptic ulcer disease [24,30-31]. The results of the current study indicated that 93.9 % of *H. pylori* isolates examined had at least one of these two virulence genes as evidenced by PCR-based molecular testing. These results were in agreement with that obtained by [32].

Our data indicated that the incidence of *H. pylori*-related diseases has been observed to be similar among men and women and no statistically significant difference in prevalence based on gender. However [33,34], reported that the rate of infection with *H. pylori*, afflict men more frequently than women studied among 556 African-Americans. In a study reported by [35] the prevalence rate among males (18.9%) was significantly higher (p<0.001) than that among females (9.0%).

The *cagA* gene was detected in 81.8% (27/33) of recovered *H. pylori* specimens which is similar to other countries [36,37]. The frequency of *cagA* gene was reported to be around 62% in a Saudi study [38] compared to 70% in Europe, 85% in Estonian and Russia, 90% in East Asia [39] and 63% in Japan [40]. The percentage of *cagA*+ genotype within each clinical outcome was significantly higher than that of*cagA*- genotype (p<0.001). However, the prevalence of *cagA*+ and *cagA*- genotypes did not differ significantly between the two clinical outcomes (p=0.658).

Likewise, the *iceA* gene was detected in 81.7 % (27/33), while 6 cases (18.8 %) were *iceA*- by PCR (Figure 1). The percentage of *iceA*+ genotypes were 92.3% (12/13) and 75% (15/20) for PUD and gastritis cases, respectively. The percentage of *iceA*+ genotype within each clinical outcome was significantly higher than that of *iceA*- genotype. The percentages of *iceA*+ genotype differed significantly between the two clinical outcomes, as *iceA*+ genotype was detected more frequently in PUD patients as compared with gastritis patients. As with *cagA* gene, there was no association between *iceA* genotypes and gender of patients.

In a study reported by [41] found that 87.4% of the positive *H. pylori* cases were *iceA*2 positive compared to only 12.6% cases positive for *iceA*1. As reported by [42,43] *iceA*1 expression is associated with a higher activity of the gastric inflammation, a condition that increases the risk for developing ulcer disease and gastric carcinoma.

Previous studies in the United States andthe Netherlands have demonstrated a strong association between *iceA*1 and ulcer disease which also proved by [30,31,41].

The prevalence of the combined *cagA* and *iceA* genotypes among the 13 peptic ulcer and 20 gastritis cases is shown in Figure 1. The percentage of *cagA*+/*iceA*+ genotype was significantly high (p<0.001) within peptic ulcer (76.9%) and also within gastritis (65%) cases. However, no association was revealed between the prevalence of the four genotypes (+ +, + -, - +, - -) and the clinical outcome by using 2x4 Fisher's exact test (p=0.498). Figure 1 shows that all of the *cagA*+ *H. pylori* specimens (n=10) that were typed from peptic ulcer cases were also found to have the *iceA*+ genotype. Of the 17 *cagA*+ *H. pylori* specimens that were typed from gastritis patients, 13 specimens had the *iceA*+ genotype. The *cagA*+ genotype, therefore, could be a predictive marker for the *iceA*+ genotype in *H. pylori* specimens isolated from peptic ulcer patients. This association was not observed within *H. pylori* specimens typed from gastritis cases.

The occurrence *iceA*1 and *iceA*2 double positive genotypes within the studied samples are shown in Table 2. Out of the 33 samples examined 19 (58%) were double positive for *iceA*1 and *iceA*2 genes. These double positives were found in 10 PUD (11/13=77%) and 8 gastritis (8/20=40%) cases. Thus, it appears that infection with multiple strains of *H. pylori* occurs more frequently in patients with PUD, compared to those with gastritis. Out of the 19 *iceA*1 and *iceA*2 double positive samples, 18 samples had the *cagA*+ genotype. Table 2 shows that *cagA*+/*iceA*1/*iceA*2 double positive genotypes occurred

doi: 10.7243/2052-6180-2-2

in 77% of PUD cases and in only 40% of gastritis cases.

These results are in agreement with those reported by [33] who found a high correlation between the *iceA*1+ and peptic ulcer disease. Also as reported by [39], all the ulcer cases (100 %) were *iceA*1 positive with statistically significant correlation (p=0.0001), while *iceA*1 allele was found in 94.6% of gastritis cases. Other studies from Asia suggested no association between *cagA* & *iceA* genotypes and peptic ulcer disease [44]. As investigated by [45] *H. pylori* genotypes are not equally distributed all over the world.

In conclusion, PCR- based genotyping should be done for high-risk patients who are infected with multi genotypes of *H. pylori* in order to prevent the development of ulcer and cancer diseases later in their life.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions

Authors' contributions	RHK	EMH	HSA
Research concept and design	✓	✓	✓
Collection and/or assembly of data	✓	✓	✓
Data analysis and interpretation	✓	✓	✓
Writing the article	✓	✓	✓
Critical revision of the article	--	✓	✓
Final approval of article	--	✓	✓
Statistical analysis	--	✓	✓

Acknowledgement
This work was supported by Taif University and A grant from the Applied Research Program-Graduate Students, King AbdulAziz for Science and Technology, Riyadh. MSc. Contract n°: 1000-12. Our thanks go to the Ministry of Health hospitals in Saudi Arabia for providing us with gastric biopsy specimens.

Publication history
Editor: Lisa Durso, United States Department of Agriculture, USA.
EIC: Todd R. Callaway, Food and Feed Safety Research Unit, USA.
Received: 08-Aug-2013 Revised: 07-Sep-2013
First Re-Revised: 22-Jan-2014 Finial Re-Revised: 01-Feb-2014
Accepted: 11-Feb-2014 Published: 17-Feb-2014

References
1. Peek RM, Jr. and Blaser MJ. **Helicobacter pylori and gastrointestinal tract adenocarcinomas**. *Nat Rev Cancer.* 2002; **2**:28-37. | Article | PubMed
2. Suerbaum S and Michetti P. **Helicobacter pylori infection**. *N Engl J Med*. 2002; **347**:1175–86. | Article
3. Cavaleiro-Pinto M, Peleteiro B, Lunet N and Barros H. **Helicobacter pylori infection and gastric cardia cancer: systematic review and meta-analysis**. *Cancer Causes Control.* 2011; **22**:375-87. | Article | PubMed
4. Ho SA, Hoyle JA, Lewis FA, Secker AD, Cross D, Mapstone NP, Dixon MF, Wyatt JI, Tompkins DS, Taylor GR and et al. **Direct polymerase chain reaction test for detection of Helicobacter pylori in humans and animals**. *J Clin Microbiol.* 1991; **29**:2543-9. | Article | PubMed Abstract | PubMed Full Text
5. Hammar M, Tyszkiewicz T, Wadstrom T and O'Toole PW. **Rapid detection of Helicobacter pylori in gastric biopsy material by polymerase chain reaction**. *J Clin Microbiol.* 1992; **30**:54-8. | Article | PubMed Abstract | PubMed Full Text
6. Liu H, Rahman A, Semino-Mora C, Doi SQ and Dubois A. **Specific and sensitive detection of H. pylori in biological specimens by real-time RT-PCR and in situ hybridization**. *PLoS One.* 2008; **3**:e2689. | Article | PubMed Abstract | PubMed Full Text
7. Rimbara E, Sasatsu M and Graham DY. **PCR detection of Helicobacter pylori in clinical samples**. *Methods Mol Biol.* 2013; **943**:279-87. | Article | PubMed
8. Menoni SM, Bonon SH, Zeitune JM and Costa SC. **PCR-Based Detection and Genotyping of Helicobacter pylori in Endoscopic Biopsy Samples from Brazilian Patients**. *Gastroenterol Res Pract.* 2013; **2013**:951034. | Article | PubMed Abstract | PubMed Full Text
9. Goodman KJ and Correa P. **Transmission of Helicobacter pylori among siblings**. *Lancet.* 2000; **355**:358-62. | Article | PubMed
10. Glynn MK, Friedman CR, Gold BD, Khanna B, Hutwagner L, Iihoshi N, Revollo C and Quick R. **Seroincidence of Helicobacter pylori infection in a cohort of rural Bolivian children: acquisition and analysis of possible risk factors**. *Clin Infect Dis.* 2002; **35**:1059-65. | Article | PubMed
11. Hussein NR, Napaki SM and Atherton JC. **A study of Helicobacter pylori-associated gastritis patterns in Iraq and their association with strain virulence**. *Saudi J Gastroenterol.* 2009; **15**:125-7. | Article | PubMed Abstract | PubMed Full Text
12. Fakhro AR, Fateha Bel D, Amin Farid IM and Jamsheer HM. **The association between Helicobacter pylori infection and lymphoid reaction in patients suffering from dyspepsia in Bahrain**. *Saudi J Gastroenterol.* 1999; **5**:129-33. | Article | PubMed
13. Nimri LF, Matalka I, Bani Hani K and Ibrahim M. **Helicobacter pylori genotypes identified in gastric biopsy specimens from Jordanian patients**. *BMC Gastroenterol.* 2006; **6**:27. | Article | PubMed Abstract | PubMed Full Text
14. Al Qabandi A, Mustafa AS, Siddique I, Khajah AK, Madda JP and Junaid TA. **Distribution of vacA and cagA genotypes of Helicobacter pylori in Kuwait**. *Acta Trop.* 2005; **93**:283-8. | Article | PubMed
15. Mahmoud RAK, Morcos HH, Hegazi AA, Abo Seif MA and ElHadidy KS. **The serological gastric biopsy: a non-endoscopical/histopathologic diagnostic approach in management of the dyspeptic patients**. *Am J Immunol*. 2006; **2**:88-96. | Article
16. Ayoola AE, Ageely HM, Gadour MO and Pathak VP. **Prevalence of Helicobacter pylori infection among patients with dyspepsia in South-Western Saudi Arabia**. *Saudi Med J.* 2004; **25**:1433-8. | Article | PubMed
17. BinSaeed AA. **Glimpse of the epidemiological research on Helicobacter pylori in Saudi Arabia**. *Saudi J Gastroenterol.* 2009; **15**:85. | Article | PubMed Abstract | PubMed Full Text
18. Martins LC, Corvelo TC, Demachki S, Araujo MT, Assumpcao MB, Vilar SC, Freitas FB, Barbosa HP, Fecury AA, do Amaral RK and Dos Santos SE. **Clinical and pathological importance of vacA allele heterogeneity and cagA status in peptic ulcer disease in patients from North Brazil**. *Mem Inst Oswaldo Cruz.* 2005; **100**:875-81. | Article | PubMed
19. Rizzato C, Torres J, Plummer M, Munoz N, Franceschi S, Camorlinga-Ponce M, Fuentes-Panana EM, Canzian F and Kato I. **Variations in Helicobacter pylori cytotoxin-associated genes and their influence in progression to gastric cancer: implications for prevention**. *PLoS One.* 2012; **7**:e29605. | Article | PubMed Abstract | PubMed Full Text
20. Arevalo-Galvis A, Trespalacios-Rangell AA, Otero W, Mercado-Reyes MM and 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. | Pdf | PubMed
21. Zhou J, Zhang J, Xu C and He L. **cagA genotype and variants in Chinese Helicobacter pylori strains and relationship to gastroduodenal diseases**. *J Med Microbiol.* 2004; **53**:231-5. | Article | PubMed
22. Proenca-Modena JL, Acrani GO and Brocchi M. **Helicobacter pylori: phenotypes, genotypes and virulence genes**. *Future Microbiol.* 2009; **4**:223-40. | Article | PubMed
23. Shiota S, Watada M, Matsunari O, Iwatani S, Suzuki R and Yamaoka Y. **Helicobacter pylori iceA, clinical outcomes, and correlation with cagA: a meta-analysis**. *PLoS One.* 2012; **7**:e30354. | Article | PubMed Abstract | PubMed Full Text

24. Yamaoka Y, Kodama T, Gutierrez O, Kim JG, Kashima K and Graham DY. **Relationship between Helicobacter pylori iceA, cagA, and vacA status and clinical outcome: studies in four different countries**. *J Clin Microbiol.* 1999; **37**:2274-9. | Article | PubMed Abstract | PubMed Full Text

25. Ando T, Peek RM, Pride D, Levine SM, Takata T, Lee YC, Kusugami K, van der Ende A, Kuipers EJ, Kusters JG and Blaser MJ. **Polymorphisms of Helicobacter pylori HP0638 reflect geographic origin and correlate with cagA status**. *J Clin Microbiol.* 2002; **40**:239-46. | Article | PubMed Abstract | PubMed Full Text

26. Yamaoka Y, Orito E, Mizokami M, Gutierrez O, Saitou N, Kodama T, Osato MS, Kim JG, Ramirez FC, Mahachai V and Graham DY. **Helicobacter pylori in North and South America before Columbus**. *FEBS Lett.* 2002; **517**:180-4. | Article | PubMed

27. Ashour AA, Collares GB, Mendes EN, de Gusmao VR, Queiroz DM, Magalhaes PP, de Carvalho AS, de Oliveira CA, Nogueira AM, Rocha GA and Rocha AM. **iceA genotypes of Helicobacter pylori strains isolated from Brazilian children and adults**. *J Clin Microbiol.* 2001; **39**:1746-50. | Article | PubMed Abstract | PubMed Full Text

28. Blaser MJ, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, Stemmermann GN and Nomura A. **Infection with Helicobacter pylori strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach**. *Cancer Res.* 1995; **55**:2111-5. | PubMed

29. Atherton JC. **CagA: a role at last**. *Gut.* 2000; **47**:330-1. | Article

30. Van Doorn LJ, Figueiredo C, Sanna R, Plaisier A, Schneeberger P, de Boer W and Quint W. **Clinical relevance of the cagA, vacA, and iceA status of Helicobacter pylori**. *Gastroenterology.* 1998; **115**:58-66. | Article | PubMed

31. Peek RM, Jr., Thompson SA, Donahue JP, Tham KT, Atherton JC, Blaser MJ and Miller GG. **Adherence to gastric epithelial cells induces expression of a Helicobacter pylori gene, iceA, that is associated with clinical outcome**. *Proc Assoc Am Physicians.* 1998; **110**:531-44. | Article | PubMed

32. Taylor NS, Fox JG, Akopyants NS, Berg DE, Thompson N, Shames B, Yan L, Fontham E, Janney F, Hunter FM and et al. **Long-term colonization with single and multiple strains of Helicobacter pylori assessed by DNA fingerprinting**. *J Clin Microbiol.* 1995; **33**:918-23. | Article | PubMed Abstract | PubMed Full Text

33. Replogle ML, Glaser SL, Hiatt RA and Parsonnet J. **Biologic sex as a risk factor for Helicobacter pylori infection in healthy young adults**. *Am J Epidemiol.* 1995; **142**:856-63. | Article | PubMed

34. Matsukura N, Onda M, Kato S, Hasegawa H, Okawa K, Shirakawa T, Tokunaga A, Yamashita K and Hayashi A. **Cytotoxin genes of Helicobacter pylori in chronic gastritis, gastroduodenal ulcer and gastric cancer: an age and gender matched case-control study**. *Jpn J Cancer Res.* 1997; **88**:532-6. | Article | PubMed

35. Sasidharan S, Lachumy SJ, Ravichandran M, Latha LY and Gegu SR. **Epidemiology of Helicobacter pylori among multiracial community in Northern Peninsular, Malaysia: effect of age across race and gender**. *Asian Pac J Trop Med.* 2011; **4**:72-5. | Article | PubMed

36. **WGO**, **World Gastroenterology Organization Global Guidelines. *Helicobacter pylori* in developing countries.** 2010; 1-15. | Pdf

37. Adeniyi BA, Otegbayo JA, Lawal TO, Oluwasola AO, Odaibo GN and Samuel CO et al. **Prevalence of *Helicobacter pylori* infection among dyspepsia patients in Ibadan, South West Nigeria**. *Afr J Microbiol Res*. 2012; **6**:3399-402. | Pdf

38. Marie MA. **Relationship between Helicobacter pylori virulence genes and clinical outcomes in Saudi patients**. *J Korean Med Sci.* 2012; **27**:190-3. | Article | PubMed Abstract | PubMed Full Text

39. Saribasak H, Salih BA, Yamaoka Y and Sander E. **Analysis of Helicobacter pylori genotypes and correlation with clinical outcome in Turkey**. *J Clin Microbiol.* 2004; **42**:1648-51. | Article | PubMed Abstract | PubMed Full Text

40. Hirai I, Sasaki T, Kimoto A, Fujimoto S, Moriyama T and Yamamoto Y. **Assessment of East Asian-type cagA-positive Helicobacter pylori using stool specimens from asymptomatic healthy Japanese individuals**. *J Med Microbiol.* 2009; **58**:1149-53. | Article | PubMed

41. Momenah AM and Tayeb MT. **Helicobacter pylori cagA and iceA genotypes status and risk of peptic ulcer in Saudi patients**. *Saudi Med J.* 2007; **28**:382-5. | Article | PubMed

42. Ashour AA, Magalhaes PP, Mendes EN, Collares GB, de Gusmao VR, Queiroz DM, Nogueira AM, Rocha GA and de Oliveira CA. **Distribution of vacA genotypes in Helicobacter pylori strains isolated from Brazilian adult patients with gastritis, duodenal ulcer or gastric carcinoma**. *FEMS Immunol Med Microbiol.* 2002; **33**:173-8. | Article | PubMed

43. Ben Mansour K, Fendri C, Zribi M, Masmoudi A, Labbene M, Fillali A, Ben Mami N, Najjar T, Meherzi A, Sfar T and Burucoa C. **Prevalence of Helicobacter pylori vacA, cagA, iceA and oipA genotypes in Tunisian patients**. *Ann Clin Microbiol Antimicrob.* 2010; **9**:10. | Article | PubMed Abstract | PubMed Full Text

44. Zheng PY, Hua J, Yeoh KG and 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. | Article | PubMed Abstract | PubMed Full Text

45. Van Doorn LJ, Figueiredo C, Megraud F, Pena S, Midolo P, Queiroz DM, Carneiro F, Vanderborght B, Pegado MD, Sanna R, De Boer W, Schneeberger PM, Correa P, Ng EK, Atherton J, Blaser MJ and Quint WG. **Geographic distribution of vacA allelic types of Helicobacter pylori**. *Gastroenterology.* 1999; **116**:823-30. | Article | PubMed

Citation:
Kadi RH, Halawani EM and Abdelkader HS. **Prevalence of H. pylori strains harbouring cagA and iceA virulence genes in saudi patients with gastritis and peptic ulcer disease**. *Microbiol Discov.* 2014; **2**:2. http://dx.doi.org/10.7243/2052-6180-2-2