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**Diversity of *Helicobacter pylori* genotypes in Iranian patients with different gastroduodenal disorders**

VaziriF *et al*., *Helicobacter pylori* genotype diversities in Iran

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**Abstract**

**AIM:** To investigate diversity of *Helicobacter pylori* (*H. pylori)* genotypes and its correlation with disease outcomes in an Iranian population with different gastroduodenal disorders.

**METHODS:** Isolates of *H. pylori* from patients with different gastroduodenal disorders were analyzed after culture and identification by phenotypic and genotypic methods. Genomic DNA was extracted with the QIAamp DNA mini kit (Qiagen, Germany). After DNA extraction, genotyping was done for *cagA*, *vacA (*s and m regions*)*, *iceA* (*iceA1,iceA2*) and *babA2* by specific primers for each allele using PCR. All patients’ pathologic and clinical data and their relation with known genotypes were analyzed by using SPSS ver. 19.0 software. χ2test and Fisher’s exact test were used to assess relationships between categorical variables. The level of statistical significance was set at *P*<0.05.

**RESULTS:** A total of 71 isolates from 177 patients with different gastroduodenal disorders were obtained. Based on analysis of the *cagA* gene (positive or negative), *vacA* s-region (s1 or s2), *vacA* m-region (m1 or m2), *iceA* allelic type (*iceA1* and i*ceA2*) and *babA2* gene (positive or negative), twenty different genotypic combinations were recognized. The prevalence of *cagA, vacAs1, vacAs2, vacAm1, vacAm2, iceA1, iceA2,iceA1+iceA2* and *babA2* were62%, 78.9%, 19.7%, 21.1%, 78.9%, 15.5%, 22.5%, 40.8% and 95.8%, respectively. Interestingly, evaluation of PCR results for *cagA* in 6 patients showed simultaneous existence of *cagA* variants according to their size diversities that proposed mixed infection in these patients. The most prevalent genotypes in *cagA* positive isolates was *cagA+/vacA s1m2/iceA1+A2/babA2*+ and in *cagA* negative isolates was *cagA-/vacA s1m2/iceA-/babA2*+. There were no relationships between the studied genes and histopathological findings (*H. pylori* density, neutrophil activity, lymphoid aggregation in Lamina propria and glandular atrophy). The strains which carry *cagA,vacAs1/m1*, *iceA2* and *babA2* genes showed significant associations with severe active chronic gastritis (*P =* 0.011, *P =* 0.025, *P =* 0.020, *P =* 0.031, respectively). The *vacA s1* genotype had significant correlation to the presence of the *cagA* gene (*P =* 0.013). Also *babA2* genotype showed the associations with *cagA* (*P =* 0.024). In the combined genotypes only *cagA+/vacAs1m1/iceA2/babA2+* genotype showed correlation to the severe active chronic gastritis (*P =* 0.025).

**CONCLUSION:** This genotyping panel can be a useful tool for detection of virulent *H. pylori* isolates and can provide a valuable guidance for prediction of the clinical outcomes.

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**Key words:** *Helicobacter pylori; cagA; vacA; iceA; babA2*

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**INTRODUCTION**

Infection with *Helicobacter pylori (H. pylori)* causes different clinical disorders such as persistent gastritis, peptic ulcers and Mucosa associated lymphoid tissue(MALT) lymphoma. Current studies suggest that *H. pylori* infection may be a crucial risk factor in the development of gastric cancer[1,2]. In this regard, this pathogen has been categorized as a group I carcino­gen by the International Agency for Research on Cancer[3]. The detailed reasons for these different clinical outcomes are unknown, but they may be related to host genetic factors, exposure to environmental factors (e.g., diet, drug usage, acidity of the stomach, and smoking) and to the bacterial genotypes[4]. *H. pylori* shows extensive genetic diversity and this variability has a crucial role in pathogenesis of this bacterium[5]. Several *H. pylori* virulence factor genes related to the risk of gastroduodenal disorders, including *cagA*, *vacA, babA* and *iceA,*have been proposed[6]. A tremendous number of studies proved that CagA and VacA producing strains are related to severe clinical outcomes[7]. In addition to *cagA* and *vacA*, the other *H. pylori* virulence factors, such as *iceA* and *babA2,* also showed such associations in some studies[8,9]. Beyond the role of these factors in progression of the disease, there are several papers which reported a relationship between failure of *H. pylori* eradication therapy and the strains’ virulence factor genotypes[10]. Analysis of genetic structure of virulence factors among the isolates from different geographic regions will provide new insights regarding the pathogenesis and treatment of *H. pylori* infection. *H. pylori* genotyping may have multiple roles including impact on the cure rates of eradication therapy[10], determination of clinical outcomes[11], tracking human migration[12,13] and recently, the prediction of progression of gastric preneoplastic lesions[14]. Distribution pattern of *H. pylori* genotypes and its correlation with disease outcome shows geographic differences. The aim of this study was to assess diversity of *H. pylori* genotypes in an Iranian population to determine genotypically more associated *H. pylori* isolates with different gastroduodenal disorders.

**MATERIALS AND METHODS**

***Clinical specimens***

Three Gastric biopsies (two were used for histological examination and one for culture) were obtained from 177 adult patients undergoing routine diagnostic endoscopy referred to endoscopy centre of Taleghani hospital of Tehran, Iran, after obtaining informed consent. All subjects were answering to questionnaire related to age, sex, gastric or duodenal peptic ulcer diseases upon endoscopy.

***Culture***

Antral or body biopsy specimens from each patient were kept in transport medium consisting of thioglycolate with 1.3 g/L agar (Merck) and 3% yeast extract (Oxoid). The endoscopic biopsy specimens were cut into small pieces, homogenized with a sterile scalpel and were smeared on the surface of Brucella agar plates supplemented with 7% horse blood and campylobacter selective supplement (Vancomycin 2.0 mg, Polymyxin 0.05 mg, Trimethoprim 1.0 mg) and Amphotericin B (2.5 mg/L). Incubation was performed in microaerophilic conditions at 37 °C for 5-7 d. Identification of *H. pylori* isolates were made by analysing colony morphology, Gram staining, oxidase, catalase and urease activities and *H. pylori* specific PCR (*glmM)*. The isolates were preserved in BHI broth containing 20% glycerol and 10% fetal calf serum and stored at -70°C.

***DNA extraction***

Genomic DNA was extracted with the QIAamp DNA mini kit (Qiagen, Germany) according to the manufacturer’s instructions. The DNA was stored at -20°C until used for molecular studies.

***H. pylori* *genotyping***

After DNA extraction, polymerase chain reactions (PCR) were performed in a volume of 25 μL containing 1x PCR buffer, 1 μM of each primers, 1 μL of genomic DNA (approximately 150 ng), 200 μM of dNTPs mix, 2 mM of MgCl2, and 0.05 U/μL Taq DNA polymerase. PCR amplifications were performed in an automated thermal cycler (AG 22331; Eppendorf, Hamburg, Germany) under the following conditions: for *vacA s/m*: 33 cycles of 1 min at 94 °C, 33 seconds at 55 °C, and 1 min at 72°C; for *cagA*: 33 cycles of 1 min at 94 °C, 1 min at 59 °C, and 1 min at 72 °C; for *iceA1/A2*: 33 cycles of 1 min at 94 °C, 40 seconds at 58 °C, and 1 min at 72 °C, and for *babA2*: 35 cycles of 1 min at 94 °C, 40 seconds at 58 °C and 1 min at 72 °C. The amplified genes were detected by electrophoresis in a 1.2 % agarose gel with ethidium bromide. Table 1 summarized the primer sequences, annealing temperatures and the expected size of the PCR products.

***Histopathological evaluation***

Sections were stained with hematoxylin and eosin to analyze *H. pylori* -related histology by an expert pathologist. Then the grade of gastritis was scored based on the updated Sydney System.

***Statistical analysis***

Data were analyzed by using SPSS ver. 19.0.0 software (IBM). χ2 test and Fisher’s exact test were used to assess relationships between categorical variables. The level of statistical significance was set at *P* < 0.05.

**RESULTS**

***Infection rates and clinical disorders***

A total of 71 isolates from 177 patients (~40%) with different gastroduodenal disorders were obtained. The *H. pylori* positive patients were included of 24 males and 47 females, with their ages ranging between 19 and 85 years (mean age, 66 years). All of the isolates showed positive results for common identification test and *H. pylori* specific PCR (*glmM)*. Most of the infected patients suffered from chronic gastritis (84.6 %), while the others showed duodenitis (9.8 %), intestinal metaplasia(2.8 %), hyperplasia(1.4%) and gastric cancer diseases(1.4%) (Table 2).

***Allelic diversities in main putative virulence markers***

**cagA genotyping:** The 400-bp PCR product indicating the presence of the *cagA* gene was obtained with 44 isolates (62%) and 27 (38%) were negative. Interestingly, evaluation of PCR results for *cagA* in 6 patients showed simultaneous existence of *cagA* variants according to their size diversities.

**vacA genotyping:** The frequency of *vacA s1*, *vacA s2*, *vacA m1* and *vacA m2* were 78.9 %, 19.7 %, 21.1 % and 78.9 % , respectively. Only one isolate was *vacA s0m2* (with no PCR product for s region).

**iceA genotyping:** Sole existence of *iceA1* genotypes were detected in 15.5 % and *iceA2* genotypes in 22.5 % of the colonized patients. Interestingly, out of the total studied samples, 40.8 % were infected with both *iceA1* and *iceA2* genotypes and 21.1% were negative for these genes.

**babA2 genotyping:** *babA2* was found in 68 of the patients (95.8% ), however three patients (4.2%) did not show this allelic variant (Figure 1).

***Correlation of*** *H. pylori* ***genotypes with pathological data, patients’ age and clinical outcome***

**Combination of genotypes:** Based on the analysis of the *cagA* gene (positive or negative), *vacA* s-region (s1 or s2), *vacA* m-region (m1 or m2), *iceA* allelic types (*iceA1* and *iceA2*) and *babA2* (positive or negative), twenty different genotypic combinations were recognized. The most prevalent genotypes in *cagA* positive isolates was *cagA+/vacAs1m2/iceA1+A2+/babA2*+ and in *cagA* negative isolates was *cagA-/vacAs1m2/iceA-/babA2*+(Figure 2).

***Helicobacter pylori* density, neutrophil activity, lymphoid aggregation in lamina propria and glandular atrophy:** There was no significant relationship between *cagA* positivity and *H. pylori* density, neutrophil activity, lymphoid aggregation in Lamina propria and glandular atrophy in the biopsies. Also no relationships were found between other genes and these histopathological findings.

**Patients’ age:** There was no significant relationship between the genotypes, clinical, pathological data and patients’ age.

**Chronic gastritis:** The gastritis was scored as severe active chronic gastritis, moderate active chronic gastritis, mild active chronic gastritis, severe chronic gastritis and moderate chronic gastritis. The strains which carry *cagA* gene showed significant associations with severe active chronic gastritis (*P =* 0.011). Also the strains which carry *vacA s1/m1* gene showed significant associations with severe active chronic gastritis (*P =* 0.025). *babA2* (*P =* 0.031) and *iceA2* (*P =* 0.020), also had significant correlation with the severe active chronic gastritis. In the combined genotypes this association was observed for *cagA+/vacAs1m1/iceA2/babA2+*genotype in the case of severe active chronic gastritis (*P =* 0.025).

**Genotype correlation:** Interestingly, the *vacA s1* genotype had significant correlation to the presence of the *cagA* gene (*P =* 0.013). Also *babA2* genotype showed this associations in *cagA* positive isolates(*P =* 0.024).

**DISCUSSION**

*H. pylori* infection usually present in 60%-80% of gastric and 95% of duodenal ulcers. However, some conditions affect infection rate of this bacterium in different geographic and socioeconomic regions. The prevalence of infection is typically higher in developing countries (greater than 80%) and lower in the developed ones (typically less than 40%)[20]. It has been demonstrated that prevalence of *H. pylori* infection in developing countries with low socioeconomic and poor management of drinking water is much higher (>80%) than that in developed countries (< 60%)[21]. In our study the recovery rate of *H. pylori* was 40% which show the improvement in the living conditions and hygiene in Iran which also reported recently[ 22].

*H. pylori* can be divided into *cagA*-positive and *cagA*-negative strains, and there is increasing evidence that infection with *cagA* positive isolates are associated with a greater risk of adverse clinical outcomes than infections with strains lacking this gene. In the current study the strains which carry *cagA* gene showed significant associations with severe active chronic gastritis. Interestingly, the prevalence of the *cagA*-positive strain differs among different countries, and more than 90% of *H. pylori* strains are *cagA* positive in East Asian countries, irrespective of clinical presentation[23]. Sasaki *et al*[24] showed that among *H. pylori* DNA-positive samples, *cagA* was detected in 45.9% from Ecuador and 20.0% from Panama. In our study the prevalence of *cagA* positive isolates is 62% which is less than other Asian countries and more than other countries (e.g. Ecuador, Panama). According to Watada *et al*[25] study, the prevalence of *cagA* was 65.5% in Colombia and 100% in Japan, which showed that the prevalence of this gene in our study is similar to the Colombian isolates. In another study conducted in Bulgaria, the prevalence of *cagA* was 84.9% which is more than our results[26]. Interestingly, we had 6 isolates which had two different size of *cagA* simultaneously, that showed occurrence of the mixed infection in these patients.

Variations of *vacA* are associated with different risks of gastrointestinal disorders. In general, *vacA* s1 and m1 genotypes produce a large amount of toxin, whereas s2 and m2 genotypes show little or no toxin production[27]. Recently, a third polymorphic determinant of vacuolating activity has been described as located between the s-region and m-region, an intermediate (i) region[28]. The frequency of the *vacA s1* and *vacA m1* genotypes in the Middle Eastern countries was 71.5% and 32.8% respectively [11], which are in concordance with our study. We didn’t detect any *vacA s2m1* genotypes in our isolates which had been reported to be rare[23]. The *vacA* s1 and m1 genotypes have been reported to be associated with the *H. pylori*-related diseases; however *vacA* s2 and m2 strains are rarely associated with peptic ulcer and gastric cancer because of their low or no-vacuolating activities[23]. Genotyping of *vacA* will be useful in screening individuals for risk factors associated with gastric cancer and peptic ulcer development. Asrat *et al*[29] showed that *vacA s1m1* genotype was the most common genotype in Ethiopian adult dyspeptic patients and also the *vacA* and *cagA* positive *H. pylori* strains were detected to a higher degree in patients with chronic active gastritis. Interestingly, similar to our results correlation of the *vacA s1* genotype with the presence of the *cagA* gene was reported by Atherton *et al*[30]. The *vacA s1m2* genotype is more common in our Iranian patients as previously described in Iran[31]. As reviewed by Suzuki *et al*[32] the predominant *vacA* genotypes in Asia, Europe and Africa is *vacA* s1m1 and their subtypes, which is in contrast to our genotypes in Iranian isolates.

In spite of the low frequency of *vacA s1m1* genotypes in our study, isolates which carry *vacA s1m1* gene showed significant associations with severe active chronic gastritis. In a review by Hosseini *et al*[33] they concluded that in contrast to *vacA*, there is no correlation between *cagA* genotype and disease status in the majority of studies conducted in Iran, but results of our study, however proposed both of these genetic markers as useful indicators for predicting clinical outcomes in the studied population.

The Meta analysis by Shiota *et al*[8] confirmed the importance of the presence of *iceA* gene for peptic ulcer, although the significance was controversial. Such different results between the *iceA* allelic types and clinical disorders could be explained by the difference in geographic regions. In our study we found significant relation between *iceA2* genotype and the clinical outcomes (severe active chronic gastritis); which was also observed by Caner *et al*[34] in Turkey. As Shiota *et al*[ 35-37] summarized in their Meta analysis most of the studies showed no association between *iceA1* and *cagA* status which is in concordance with our study. Interestingly, the prevalence of mixed genotype *iceA1*+*iceA2* (40.8%) in our study, was higher than other studies which had detected this mixed genotypes. So this high prevalence with mixed genotypes makes it difficult to analyze potential relationships between the presence of each *iceA* allelic variant and clinical outcomes. *babA2* genotype was frequently found in *H. pylori* strains in our study (95.8%),that was associated with severe active chronic gastritis. Although this genotype showed significant correlation with the existence of *cagA,* but no significant correlation was observed with other virulence factors such as *vacA s1/s2* ,*vacA m1/m2* and *iceA1/iceA2*. Chomvarin *et al*[38] detected the *babA2* gene in 92% (103/112) of the Thai patients, which is almost similar to our results; while in another study conducted in Cubathe prevalence of *babA2* gene was lower (82.3 %)[39].

In the combination of genotypes, we observed twenty different genotypes which showed vast diversities in the *H. pylori* isolates in our study. Interestingly there was no any significant association between these combined genotypes and clinical outcomes, except for *cagA+/vacAs1m1/iceA2/babA2+*genotype which showed significant association with severe active chronic gastritis.

Genotypes of *H. pylori*, especially *cagA* and *vacA*, are reported to be crucial factors determining the cure rates. So to select an *H. pylori* eradication regimen, we need to consider *H. pylori* genotypes[10]. *H. pylori* genotypes distributions and their correlations with disease outcomes had shown geographical differences. In this regard Yamaoka *et al*[7] reviewed that within East Asia, where the incidence of gastric cancer is high; *vacA m1* genotype is dominant, whereas in southern parts where the gastric cancer incidence is low, the *m2* genotype, which we observed in our study, is predominant. Dabiri *et al*[31] showed that there was no statistically an association between the *vacA*, *cagA* and *cagE* status with clinical outcomes in Iranian patients and recommended that other different markers may be more useful for this analysis. In comparison, in the current study, genotyping on the basis of *cagA*, *babA2*, *vacA* and *iceA* were considered as useful tool for predicting the clinical outcomes. Therefore analyzing the multiple virulence factors of *H. pylori* (*cagA*, *vacA*, *iceA* and *babA2*) might enable us to predict the patient’s clinical outcome in Iranian patients. This prediction could be more accurate when accompanied by the impacts of environmental factors and host genetic polymorphism such as interleukin-1 receptor antagonist (IL-1RN) gene polymorphism[37]. Nowad concurrent genotyping of *H. pylori* virulence markersand host factors is becoming increasingly crucial in the prediction of the diseases outcomes[40].

In conclusion our results showed that most of the *H. pylori* isolates were highly virulent on the basis of the main clinically allelic variants in three or four virulence factors they could carry. The Iranian isolates predominantly possessed different genotypes which showed vast diversities. Significant association of the noted genotypes with sever active chronic gastritis proposed this genotyping panel as a suitable tool for detection of virulent *H. pylori* isolates that could provide a valuable guidance for prediction of the clinical outcomes.

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**COMMENTS**

***Background***

Infection with *Helicobacter pylori* (*H. pylori)* causes diverse clinical outcomes such as persistent gastritis, peptic ulcers, MALT lymphoma and gastric cancer. One of the reasons of these different clinical outcomes is genetic diversity of *H. pylori*; therefore determination of pattern of *H. pylori* genotypes and its correlation with disease outcome, which shows geographic differences, is crucial.

***Research frontiers***

The *H. pylori* genotyping may have multiple roles including prediction of clinical outcomes, impact on the *H. pylori* infection therapy, tracking human migration, and recently, the prediction of progression of gastricpreneoplastic lesions. Therefore genotyping of *H. pylori* can be a valuable and multifunctional tool in the clinical field.

***Innovations and breakthroughs***

In the majority of the previous studies the researchers were not able to detect any significant relationship between their genotyping panels and clinical outcomes for *H. pylori* infections. Most of these studies had been used few genetic markers. In order to overcome this disadvantage The authors have chosen greater numbers of *H. pylori* genetic markers for studying this association.

***Applications***

The genotyping panel which contains eight important genetic markers can be served as a useful tool for typing of *H. pylori* isolates and to some extent prediction of clinical outcomes.

***Peer review***

This is an epidemiological paper with statistical analysis, dealing with the important question of association between certain *H.pylori* genotypes and specific pathologies, and with the problem of predictive value of *H.pylori* infection genotyping. Although the question of *H.pylori* genetic diversity in Iranian population was already addressed in at least two publications, in the submitted manuscript this issue is dissected in fine details and using quite extensive clinical material, thus providing novel and more reliable data.

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|  |

**Table 1** **Primers used in this study**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Gene** | **Primers (5′ 3′)** | **PCR product (bp)** | **Annealing temperature (°C)** | **Ref.** |
| ***vacA (s1/S2)*** | VA1F: ATGGAAATACAACAAACACAcVA1R: CTGCTTGAATGCGCCAAAC | 259-286 | 55 | 6 |
| ***vacA (m1/m2)*** | VACm1m2F: CAATCTGTCCAATCAAGCGAGVACm1m2R: GCGTCAAAATAATTCCAAGG | 567-642 | 55 | 15 |
| ***cagA*** | CagAF: AATACACCAACGCCTCCAAG CagAR: TTGTTGCCGCTTTTGCTCTC  | 400 | 59 | 16 |
| ***iceA1*** | iceA1F: TATTTCTGGAACTTGCGCAACCTGATM.Hpy1R: GGCCTACAACCGCATGGATAT | ~900 | 58 | 17 |
| ***iceA2*** | iceA2 F: CGGCTGTAGGCACTAAAGCTAiceA2 R: TCAATCCTATGTGAAACAATGATCGTT | ~800 | 58 | 17 |
| ***babA2*** | babA2F:CCAAACGAAACAAAAAGCGTbabA2R:GCTTGTGTAAAAGCCGTCGT | 271 | 58 | 18 |
| ***glmM*** | GlmM2-F GGATAAGCTTTTAGGGGTGTTAGGGGGlmM1-R GCTTACTTTCTAACACTAACGCGC | 296 | 52 | 19 |

**Table 2** Association of combined genotypes with pathological conditions in *Helicobacter pylori* isolates

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Combination of genotypes** | **SCG** | **SACG2** | **MACG** | **MiACG** | **MCG** | **H** | **M** | **GC** | **D** | **Total** | ***P* value1** |
| *cagA+/vacAs1m2/iceA1+iceA2/babA2+* | 1 | 12 | 2 | 0 | 0 | 0 | 1 | 0 | 1 | 17 |  |
| ***cagA+/vacAs1m1/ iceA2/babA2+*** | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 4 | 0.0252 |
| *cagA+/vacAs1m2/iceA1 /babA2+* | 0 | 3 | 1 | 0 | 0 | 0 | 1 | 0 | 2 | 7 |  |
| *cagA+/vacAs1m1/iceA1+iceA2/babA2+* | 0 | 6 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 8 |  |
| *cagA+/vacAs2m2/iceA1+iceA2/babA2+* | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |  |
| *cagA+/vacAs0m2/ iceA2/babA2+* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |  |
| *cagA+/vacAs1m2/ iceA2/babA2+* | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |  |
| *cagA+/vacAs2m2/iceA1 /babA2+* | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |  |
| *cagA+/vacAs2m2/iceA- /babA2+* | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |  |
| *cagA+/vacAs1m2/iceA-\_/babA2+* | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |  |
| *cagA-/vacAs1m2/iceA­-/babA2+* | 0 | 3 | 2 | 0 | 1 | 0 | 0 | 0 | 1 | 7 |  |
| *cagA-/vacAs1m1/iceA­-/babA2+* | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 2 |  |
| *cagA-/vacAs2m2/iceA­2/babA2+* | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 3 |  |
| *cagA-/vacAs1m2/iceA­2/babA2+* | 0 | 2 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 5 |  |
| *cagA-/vacAs2m2/iceA­1/babA2+* | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |  |
| *cagA-/vacAs1m1/iceA2/babA2+* | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |  |
| *cagA-/vacAs2m2/iceA­-/babA2+* | 0 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |  |
| *cagA-/vacAs1m2/iceA­1+iceA2/babA2+* | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |  |
| *cagA-/vacAs1m2/ iceA­1+iceA2/babA2-* | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |  |
| *cagA-/vacAs2m2/ iceA­1 /babA2-* | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 2 |  |
| Total | 2 | 39 | 13 | 1 | 5 | 1 | 2 | 1 | 7 | 71 |  |

SCG: Severe chronic gastritis; SACG: Severe active chronic gastritis; MACG: Moderate active chronic gastritis; MiACG: Mild active chronic gastritis; MCG: Moderate chronic gastritis; H:Hyperplasia; M:Metaplasia; GC: Gastric cancer; D:Duodenitis.

1Only *P*<0.05 are indicated; 2This *P* value is related to SACG.

1 2 3 4 5 6 7

300bp

500bp

1000bp

Figure 1 Polymerase chain reaction products of the main putative virulence markers. Lane 1: DNA ladder mix; Lane 2: *vacA s1m1* genotype; Lane 3: *vacA s1m2* genotype; Lane 4: *vacA s2m2* genotype; Lane 5: *iceA1+iceA2* genotype; Lane 6: *babA2* genotype; Lane 7: *cagA* genotype

**A**

**B**

**Figure 2** **The frequency of combined genotypes.** A: Combined *vacA*, *iceA* and *babA2* genotypes in 44 *cagA* positive isolate; B: Combined *vacA*, *iceA* and *babA2* genotypes in 27 *cagA* negative isolates.