Genotyping and Association of the Presence of *Helicobacter Pylori* in Dental Plaque and Gastric Biopsy Specimens in Dyspeptic Patients by PCR-RFLP Technique in Southwest of Iran

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Abstract

The oral cavity can act as an extra gastric reservoir for *H. pylori*, and also the presence of the bacteria in the oral cavity is associated with a higher risk of dental caries development. The aim of this study was to determine the genotype and evaluate the association of the presence of *H. pylori* in dental plaque and gastric biopsy specimens in dyspeptic patients in Ahvaz, Southwest of Iran.

In this study, 106 patients with recruited dyspeptic complaints were selected and from each patient, two gastric antral biopsy specimens and two dental plaques were examined. The presence of *H. pylori* was identified by the Rapid Urease Test (RUT) and the amplification of *ureAB* and *16S rRNA* genes. Also, to verify a hypothetical mouth-to-stomach infection route, the enzymatic digestions of three genes of *cagA*, *vacA*, and *ureAB* in *H. pylori* strains isolated from dental plaques and stomach samples were compared for each same case.

H. pylori was found in the stomach of 52.8% (56 /106) and the dental plaques of 17.9% (19/106) of the studied cases. On the other hand, *H. pylori* was recognized in the stomach of all 19 cases with oral colonization. Following a combination of restriction fragment length polymorphism (RFLP) patterns of these three known genes on stomach and dental plaque samples, 14 and 11 unique patterns were seen, respectively. However, for all *H. pylori*-positive cases (19), the comparison of RLFP patterns of these genes in the dental plaque and gastric biopsy specimens was different for the same case. This study showed, no significant association was observed between the presence of *H. pylori* in dental plaque and the stomach of the same case.

Introduction

Helicobacter pylori have been known as one of the most common agents of human bacterial infections. This bacterium is located under the gastric mucous layer, adjacent to the gastric epithelial cells. Infection caused by this bacterium is now recognized as a serious transmissible infectious disease (Veiga et al. 2015).

H. pylori infections are common in areas with low levels of social-economic health (Goh et al. 2011). Despite many investigations, the exact route of infection transmission of this microorganism is still unknown. However, two different modes are suggested: oral-oral and fecal-oral transmission (Umeda et al. 2003).

In addition to the stomach, *H. pylori* has also been isolated from the saliva and feces of the human as a likely reservoir of it (Momtaz et al. 2012). Since the oral cavity can act as an extra gastric reservoir for *H. pylori*, it may lead to recurrent gastric infection (Al Asqah et al. 2009). On the other hand, Moseeva et al. (2010) found a relationship between the colonization of this bacterium in the oral cavity and the risk of dental caries development. In contrast, Olivier et al. (Olivier et al. 2006) and Chitsaz et al. (Chitsazi et al. 2006) failed to find *H. pylori* in the dental plaque of patients with gastric infections. These controversial results may be due to several factors including the variations in the study population, sampling

procedures, and methodologies used for the detection of *H. pylori* in dental plaques. There are several diagnostic tests for *H. pylori* in dental plaques, including rapid urease test (RUT), polymerase chain reaction (PCR), immunoassay, cytology, and bacterial culture. In general, the detection rate of *H. pylori* reported in studies, using RUT was higher than other tests. The lowest detection rate was when the microbial culture was used to detect the presence of this bacterium in dental plaque (Anand et al. 2014).

Several virulence factors have been identified in *H. pylori* that can develop the severity of pathogenicity. Among them, cytotoxin-associated gene A (*cagA*) and vacuolating cytotoxin A (*vacA*) have been mentioned as the two main virulence factors of *H. pylori* that are involved in the pathogenesis of different strains. The *cagA* gene is located within a 40-kilobases DNA fragment known as the *cag* pathogenicity island (*cag* PAI). Approximately, more than 60% of *H. pylori* strains carry this gene. This gene is associated with increased production of interleukin 8, mucosal inflammation, and an increased risk of peptic ulcer (Palframan et al. 2012; Erzin et al. 2006). The *vacA* is a housekeeping gene that can induce vacuolation and apoptotic processes in epithelial cells, as well as immunosuppressive effects on some immunological cells (Palframan et al. 2012).

In this study, to investigate the hypothesis of oral to-stomach transmission in *H. pylori* infections, it is necessary to compare the RFLP patterns of several *H. pylori* genes between the mouth and stomach for the same case.

In our study, three genes of *cagA*, *vacA*, and *ureAB* were selected for these analyses. To our knowledge, no sufficient research has been done on the detection of *H. pylori* in dental plaque and gastric biopsy specimens isolated from patients with dyspepsia in Ahvaz, Southwest of Iran. Hence, the present study aimed to determine the genotype and investigate *H. pylori* in dental plaque and gastric biopsy specimens, as well as the evaluation of an association between the colonization of *H. pylori* in dental plaque specimens and gastric biopsy.

Materials And Methods

Study population and sampling

In this study, specimens were collected from 106 patients with dyspeptic complaints who were referred to the teaching hospital of Imam Khomeini, Ahvaz, Iran. Initially, two biopsy specimens from each patient were taken by a gastroenterologist from antral, within 5 cm of the pylorus. The exclusion criteria were as follows: treatment with any antibiotics, proton pump inhibitors, H2 blockers, or bismuth compounds during one month preceding this study, recent use of non-steroidal anti-inflammatory drugs, periodontal therapy within the past year, and signs of severe periodontal infections within the preceding 6 months (Liu et al. 2009).

We retained one gastric biopsy specimen for RUT and the other was stored at -70°C for DNA extraction. For performing RUT, the samples were inoculated in the urea broth medium (Sigma Co.). The needed time for a positive test depends on the concentration of *H. pylori* and temperature. Although most RUT will be

change positive within 120-180 minutes, for the negative tests and increased sensitivity, it is best to read the test at 24 hours, so in this study, the results were interpreted after one overnight.

On the other hand, two dental plaque samples were taken from each patient before the endoscopic examination. To remove the remaining foods, each patient was requested to wash his/her mouth with normal saline before sampling. Dental floss was used to completely clean the interdental spaces. The samples were transported in the tubes containing digestion buffer [100 mM NaCl, 10 mM Tris-HCl (pH 8.0), 250 mM ethylene diamine tetra acetic acid (EDTA) (pH 8.0), and 1% sodium lauryl sarcosine] on the day of sampling (Momtaz et al. 2012). One of these samples was used for screening *H. pylori* by RUT and the other was stored at -70°C for DNA extraction and PCR.

DNA extraction

The gastric biopsy and dental plaque samples were crushed and mixed by a vortex. Then, the mixtures were centrifuged at 12000 x g for 3 min. A volume of 200 µl of suspended pellets in normal saline was transferred to a 1.5 ml microtube. The genomic DNA was extracted using a High Pure PCR Template Preparation Kit (Roche Diagnosis, Mannheim, Germany) according to the protocol of DNA extraction from mammalian tissues. The concentration of the extracted DNA was measured at 260 nm, using a Nanodrop instrument (Thermo Scientific, USA) and gel electrophoresis. DNA purity was acceptable when the light absorption ratio at 260 nm to 280 nm was between 1.5 and 2.

Identification of *H. pylori* using PCR

Amplification of *ureAB* (encoding urease enzyme that is conserved in all *H. pylori* isolates) and *16S rRNA* genes were performed for identification of *H. pylori* (Rasmussen et al. 2012; Han et al. 1998). The sequence of primers used is shown in Table 1. For each gene, a uniplex PCR reaction was prepared in a volume of 25 µL. The master mixture consisted of 0.02 Units of Taq DNA polymerase, 1.5 mM MgCl₂, 0.4 µM of each primer, 1x PCR buffer, 25 ng DNA template, and distilled water up to a final volume of 25 µl.

Table 1
The sets of the used primers in this study

Gene target	Primer 5' to 3'	Amplicon size	Ref.
<i>16S rRNA</i>	F- GGAGGATGAAGGTTTTAGGAT R-TCGTTGCGGGACTTAACCCAA	150bp	Teoman et al.
<i>vacA</i>	F- GCTTCTCTTACCACCAATGC R-TGTCAGGGTTGTTCAACCATG	1162bp	Madinier et al.
<i>cagA</i>	F-AGTAAGGAGAAACAATGA R-AATAAGCCTTAGAGTCTTTTTGGAA	1320bp	Madinier et al.
<i>ureAB</i>	F- AGGAGAATGAGATGA R- ACTTTATTGGCTGGT	2420bp	Madinier et al.

The amplification reaction was carried out in Mastercycler Nexus Thermal Cycler Gradient (Eppendorf, Hamburg, Germany) with an initial denaturation step at 94°C for 5 min, followed by 35 cycles with the following profiles: for *16S rRNA*, 94°C for 1 min, 59°C for 1 min, and 72°C for 1 min; for *vacA*, 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min; for *cagA*, 94°C for 45 s, 50°C for 45 sec, and 72°C for 45 s; and for *ureAB*, 94°C for 1 min, 50°C for 1 min, and 72°C for 2 min (Rasmussen et al. 2012; Han et al. 1998). After electrophoresis of PCR products and staining of 2% gel agarose with ethidium bromide, the amplicons were observed and photographed using an ultraviolet light gel documentation system (Viber-Germany).

Restriction Fragment Length Polymorphism (RFLP) analysis

The *vacA*, *cagA*, and *ureAB* genes fragments were amplified by PCR and then were digested with *HaeIII* and *Sau3AI* for *ureAB*, *HhaI*, and *HphI* for *vacA* and *HinfI* for *cagA* for 3 hours at 37°C in the appropriate buffers suggested by the supplier (Fermentas, US). The digested products of each gene target were electrophoresed on 2% agarose gel stained with ethidium bromide (Han et al. 1998).

Statistical analysis

The descriptive statistics and chi-square test were performed in SPSS version 16.00 and a significance level of $p < 0.05$ was used in this study.

Results

A total of 106 gastric biopsy and dental plaque specimens from patients with dyspeptic complaints were evaluated. Of these patients, 88 cases were men and 18 cases were women. The patient's mean age was 33.9 ± 2.07 years. The patients were classified into four main groups based on clinical diagnosis including 58 cases with gastritis (54.7%), 35 cases with peptic ulcer (33%), 11 cases with duodenum ulcer (10.4%), and 2 cases of asymptomatic.

In this study, *H. pylori* was recognized in the stomach of 52.8% (56/106) and the dental plaques of 17.9% (19/106) of cases. On the other hand, *H. pylori* was found in the stomach of all 19 cases with dental plaque colonization. All patients with *H. pylori* colonization in dental plaque and gastric biopsy had positive results in both molecular identifications of *use* and *16S rRNA* genes and RUT (Figure1).

On the other hand, among 56 gastric biopsy specimens containing *H. pylori*, 35 cases (62.5%) had the *CagA* gene (Figure1). According to our results, the highest prevalence of the *CagA* gene belonged to patients with gastritis (20 of 28), followed by peptic ulcer (12 of 21) and duodenum ulcer (3 of 7). Also, the frequency of the *CagA* gene in dental plaque specimens was 63.1 % (12 of 19). Following enzymatic digestion of the *vacA* gene via *HhaI* and *HphI* (Figure 2a) and the *cagA* gene via *HinfI* (Figure2b) on DNA extracted from gastric biopsy and dental plaque specimens, two RFLP patterns of 3 and 4 bands for *vacA* gene and two RFLP patterns of 1 and 3 bands for *cagA* gene were visually detected. The RFLP patterns of the *vacA* and *cagA* genes have been shown in Table 2.

Table 2 The enzymatic RFLP patterns for *vacA* and *cagA* genes

Gene			Gene		
RFLP pattern	<i>vacA</i>		RFLP pattern	<i>cagA</i>	
	Dental n=19(%)	Stomach n=56(%)		Dental n=12(%)	Stomach n=35(%)
3 bands (74, 392, 704 bp)	9(47.4)	36(64.3)	1 bands (%) (1320bp)	3 (25)	10(28.6)
4 bands (50, 180, 293, 710)	10(52.6)	20(35.7)	3 bands (%) (254, 384, 712 bp)	9(75)	25(71.4)
Total	19(100)	56(100)	Total	12(100)	35(100)

On the other hand, due to the enzymatic digestion of the *ureAB* gene (Figure 2c) by *HaeIII*, four RFLP patterns of 3, 4, 5, and 6 bands were visually recognized. The RFLP patterns of the *ureAB*, *vacA*, and *cagA* genes in the stomach samples are shown in Table 3. According to the data, we recognized 14 unique patterns.

According to Table 4, by combining the RFLP patterns of these three genes in stomach and dental plaque specimens for the same case, 14 and 11 unique patterns were seen, respectively.

Table 3 The RFLP pattern of *ureAB*, *cagA*, and *vacA* genes on stomach specimens**Table 4** The combination of RFLP patterns of *ureAB*, *cagA*, and *vacA* genes on stomach and dental plaque specimens

However, for all cases with positive *H. pylori* diagnosis, a combined analysis of the RFLP patterns of these three genes was different in the dental plaque with the gastric biopsy samples for the same case. According to the data, there was no association between *H. pylori* colonization in the oral cavity and stomach for the same case, because the comparison of fingerprint patterns due to enzymatic digestion three genes (*cagA*, *vacA*, and *ureAB*) showed that these patterns were different.

Discussion

Patient Number	Stomach		
	<i>ureAB</i>	<i>Acag</i>	<i>Avac</i>
1	6bands	none	3bands
2	5bands	none	3bands
3	5bands	3bands	4bands
4	4bands	3bands	4bands
5	5bands	3bands	4bands
6	6bands	3bands	3bands
7	5bands	3bands	4bands
8	4bands	1band	4bands
9	3bands	3bands	4bands
10	6bands	1band	3bands
11	6bands	3bands	3bands
12	3bands	3bands	3bands
13	6bands	3bands	3bands
14	4bands	1band	3bands
15	3bands	3bands	3bands
16	6bands	3bands	4bands
17	4bands	3bands	4bands
18	6bands	3bands	3bands
19	5bands	none	3 bands
20	4bands	none	4bands
21	6bands	none	3bands
22	5bands	none	3bands
23	4bands	3bands	4bands
24	5bands	3bands	4bands
25	6bands	none	3bands
26	3bands	3bands	5bands

In previous, it seemed that the human stomach is only the reservoir for *H. pylori* colonization until the detection of this bacterium was reported from some dental plaques, oral cavity, and saliva. However, the recovery of *H. pylori* from the oral cavity is a controversial issue. Moreover, most attempts for isolation of this bacterium from the oral cavity by culture were encountered with failure (Umeda et al. 2003). Because *H. pylori* has a viable, non-cultivable coccoid form, several investigators have proposed that *H. pylori* in this form may survive in the mouth and is only detectable by non-culture methods such as PCR or RUT (Han et al. 1998).

The RUT, as an indirect test that can detect the presence of *H. pylori*, has advantages over serology because it can only detect active *H. pylori* infection. To obtain a positive RUT result, there must be approximately 10^5 bacteria in the sample. Since in our study, all patients with *H. pylori* colonization in the stomach and dental plaque (19 cases), had the same positive results for both RUT and PCR, therefore, it is concluded that the sensitivity of both tests is the same. In agreement with our study, some researchers also demonstrated that RUT sensitivity is greater or similar to PCR. Khalifehgholi et al. (2013) and Souod et al. 2013 have shown that the sensitivity of RUT and PCR was 95.6% and 93.5%, respectively. However, Toeman et al. (2007) recognized PCR as a more sensitive method rather than RUT for the detection of *H. pylori*. Moreover, RUT is a specific method for the detection of *H. pylori* in gastric biopsy specimens, but some researchers have questioned the validity of RUT for the diagnosis of *H. pylori* in oral specimens

27	4bands	3bands	4bands	(Madinier et al. 1997; Dowsett et al. 2003).
28	5bands	none	3bands	These controversial results can be explained by the fact that although <i>H. pylori</i> is the only urease-positive bacterium known in the gastric cavity, other urease-positive bacterial species, such as <i>Streptococcus</i> species, <i>Haemophilus</i> species, and <i>Actinomyces</i> species, also reside as part of the normal oral flora. However, according to some reports, only <i>H. pylori</i> can produce large amounts of urease during a short time, as 20 min, while delayed urease-producing bacteria do not have positive results within one hour (Anand et al. 2014). Also, Gürbüz et al. (2003) reported that RUT has a sensitivity of 89.7% and a diagnostic accuracy of 86.7% for the detection of <i>H. pylori</i> in dental plaque.
29	5bands	3bands	4bands	
30	6bands	3bands	3bands	
31	6bands	none	3bands	
32	3bands	3bands	5bands	
33	6bands	3bands	3bands	
34	6bands	none	3bands	
35	5bands	3bands	4bands	
36	4bands	none	4bands	
37	3 bands	4bands	3 bands	
38	5bands	none	3bands	
39	5bands	3bands	4bands	
Table 3 (continued)				In the present study, like many PCR assays (Han et al. 1998), we detected <i>H. pylori</i> by amplifying both urease and <i>16S rRNA</i> genes in gastric biopsy and dental plaque specimens. We identified <i>H. pylori</i> in the stomach 52.8% (106.56) cases using the PCR method.
40	3bands	3bands	5bands	
41	6bands	none	3bands	
42	5bands	3bands	4bands	
43	4bands	none	4bands	
44	3bands	4bands	3 bands	According to some reports, the frequency of <i>H. pylori</i> in dyspeptic patients in other regions of Iran using PCR was high and ranged from 48 to 85% (Roshani et al. 2014; Souod et al. 2015 and Pajavand et al. 2015). This difference in rates can be explained by the socio-economic situation population density in different regions of Iran, different methods in collecting, and samples processing, and primers used in PCR (Momtaz et al. 2012).
45	6bands	none	3bands	
46	3bands	3bands	5bands	
47	4bands	none	4bands	
48	6bands	3bands	3bands	
49	5bands	none	3bands	
50	3bands	4bands	3 bands	
51	4bands	none	4bands	On the other hand, we found <i>H. pylori</i> simultaneously in the dental plaques and the gastric biopsies of 19 (17.9%) cases. Similar to our study, Eskandari et al. (2010) in Iran (in 17.4% of cases) and Teoman et al. (2007) in
52	5bands	3bands	4bands	
53	6bands	none	3bands	
54	3bands	3bands	5bands	

55	4bands	none	4bands	China, (in 25.4% of cases) found <i>H. pylori</i> simultaneously in both the stomach and dental plaque samples.
56	4bands	none	4bands	

Patient Number	Stomach			Denta plaque			In contrast to our study, Momtaz et al. (2012) did not find any <i>H. pylori</i> in the dental plaques of patients with <i>H. pylori</i> gastritis, while they detected this bacterium in 77.6% of gastric biopsies, 10.7% of saliva, and 71.6% of stool samples. These differences in the prevalence of <i>H. pylori</i> may be due to variations in sampling techniques, different primer sets used in PCR, or some differences in the study population. In
	<i>ureAB</i>	<i>Acag</i>	<i>Avac</i>	<i>ABure</i>	<i>Acag</i>	<i>Avac</i>	
1	6bands	none	3bands	3bands	3bands	3bands	In contrast to our study, Momtaz et al. (2012) did not find any <i>H. pylori</i> in the dental plaques of patients with <i>H. pylori</i> gastritis, while they detected this bacterium in 77.6% of gastric biopsies, 10.7% of saliva, and 71.6% of stool samples. These differences in the prevalence of <i>H. pylori</i> may be due to variations in sampling techniques, different primer sets used in PCR, or some differences in the study population. In
2	5 bands	none	3bands	5 bands	3 bands	4 bands	
3	5bands	3bands	4bands	4bands	none	3bands	
4	4bands	3bands	4bands	6bands	1band	3bands	
5	5bands	3bands	4bands	5bands	none	3bands	
6	6bands	3bands	3bands	4bands	none	4bands	
7	5bands	3bands	4bands	5bands	none	3bands	
8	4bands	1band	4bands	5bands	3bands	4bands	
9	3bands	3bands	4bands	4bands	3bands	3bands	
10	6bands	1band	3bands	5bands	3bands	3bands	
11	6bands	3bands	3bands	4bands	none	4bands	
12	3bands	3bands	3bands	5bands	none	4bands	
13	6bands	3bands	3bands	5 bands	3 bands	3bands	
14	4bands	1band	3bands	3 bands	3bands	3 bands	
15	3bands	3 bands	3bands	4bands	3bands	3 bands	
16	6bands	3bands	4bands	5bands	3bands	4bands	
17	4bands	3bands	4bands	5bands	none	3bands	
18	6bands	3bands	3bands	5bands	none	4bands	
19	5 bands	none	3 bands	5bands	none	4bands	

general, there is less *H. pylori* colonization in the oral cavity than in the stomach. The low rate can be explained by the two reasons: 1) suppressor effects of oral microbiota producing bacteriocin-like inhibitory proteins against *H. pylori* strains and 2) hiding *H. pylori* in yeast such as *Candida spp.* in the oral cavity (Moseeva et al. 2010).

In our study, the frequency of the *CagA* gene in the dental plaque and gastric biopsy specimens with *H. pylori*-positive results were 52.6% and 63%, respectively. The prevalence of *cagA*-positive *H. pylori* strains was different from one geographic region to another, including 94% in Malaysia (Ramelah et al. 2005),

78% in Turkey (Saribasak et al. 2004) and 42.3% in Morocco (Alaoui et al. 2012). In addition, the prevalence of this gene was different in other regions of Iran, including 68% in Tehran (Dabiri et al. 2009), 92% in Shahrekord (Souod et al. 2013) and 76.6% in Shiraz (Kamali-Sarvestani et al. 2006).

In the present study, the number of *cagA*-positive *H. pylori* strains was more in patients with gastritis. However, Alaoui et al. (2012) and Eskandari et al. (2010) demonstrated a higher prevalence of this gene among the patients with peptic ulcer (50%) and gastric cancer (85%), respectively.

In this study, we found 14 and 11 unique patterns of RFLP in the stomach and dental plaque samples, respectively. In addition, to find a correlation between the colonization with *H. pylori* in the dental plaque and the stomach for the same case, the RFLP analyses of *vacA*, *cagA*, and *ureAB* genes were performed on the DNA extracted from the dental plaque and gastric biopsy samples. According to these analyses, the different RFLP patterns were seen for the same case. Because for each similar case, a combination of these RFLP patterns was not present in both samples (dental plaque and gastric biopsy), we concluded that there are no correlations between *H. pylori* colonization in dental plaque and stomach. In other words, in our study, the dental plaque was not considered as a likely reservoir for re-infection of the gastric with *H. pylori*.

However, in contrast to our study, Oshowo et al. (1998) demonstrated that the RFLP patterns of dental plaque and stomach samples of 13 out of 15 patients were identical for the same case. With regard that, these researchers used only one restriction endonuclease, it is difficult to prove a correlation between *H. pylori* colonization in dental plaque and the stomach for the same case.

One of the main limitations of our study was the lack of isolation of *H. pylori* by bacterial culture. Therefore, we could not determine the genotypes of the *vacA* and *cagA* genes.

Conclusion

We found a high prevalence of *H. pylori* in the dental plaque and gastric specimens. RUT was a sensitive method as same as PCR for the detection of *H. pylori*. According to the RFLP analyses on the dental plaque and gastric specimens, we showed that there was no significant correlation between the colonization of *H. pylori* in the dental plaque and stomach. However, because insufficient of sample size, our results could not confirm the dental plaque as a likely reservoir of *H. pylori* infection in the human stomach.

Study Limitation

In Fig. 1 and Fig. 2a, positive and negative controls have not been used, while they are needed in these Figures.

Declarations

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Authors` contributions

All authors have participated in the design, data collection, laboratory testing, drafting or revision of the article, have approved the final version for publication, and agree to be accountable in all aspects of the work.

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Availability of data and material

The data generated or analyzed during this study are included in this article.

Ethical approval

This research was approved by the ethics committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (Ethic of Number: IR.AJUMS.REC. 1393.224).

Conflict of Interest

The authors declare that they have no conflict of interest.

Consent for publication

All authors agree to publish.

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Figures

Figure 1

Lane 1: Size marker of 100bp; lanes 2 and 3: PCR product of 16S rRNA (150 bp), lanes 4: PCR product of *vacA* (1162 bp), lanes 5 and 6: PCR product of *cagA* (1320 bp), lanes 7 and 8: PCR product of *ureAB* (2420 bp)

Figure 2

RFLP pattern of *vacA*, *cagA* and *ureaAB* genes on agarose gel 2 % . Part2 (a): The RFLP patterns of *vacA*; lane 1: PCR product (1162bp), lanes 2 and 3: the RFLP pattern of 3 bands (74, 392, 704 bp), lanes 4, 5

and 6: the RFLP pattern of 4 bands (50, 180, 293, 710 bp), lane L: Size marker of 1000bp; Part 2 (b): The RFLP patterns of *cagA*; lanes 1 and 3: PCR product (1320bp), lane 2: the RFLP pattern of 3 bands (254, 384, 712 bp), lane L: Size marker of 100bp; Part 2 (c): The RFLP patterns of *ureAB*; lane1: the RFLP pattern of 5 bands: (302, 373, 482, 550, 803 bp), lane 2 : the RFLP pattern of 4 bands: (422, 512, 912, 1151bp), lane 3: the RFLP pattern of 6 bands (182, 315, 381, 537, 845, 1213 bp); lane 4: the RFLP pattern of 3 bands: (650, 1152, 1284bp), lane L: Size marker of 100bp