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EXPERIMENTAL WORKS

# Are Non-Pylori Helicobacters Present in the Human Oral Cavity?[1]

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**Abstract—Introduction and objective:** *Helicobacter pylori* (*H. pylori*) is a human gastric pathogen. Because of presence of *H. pylori* oral cavity, there is a possibility of *H. pylori* transmission by oral-oral route. In a cross-sectional study, the presence of some virulence factors of *H. pylori* and also non-pylori Helicobacters in dental plaque samples of participants was investigated. **Methods:** The samples were collected from at least two teeth surfaces. DNA of the samples was extracted using specific kit. The presence of *dupA* (*jhp0917* and *jhp0918*) and *babA2* genes and also *Helicobacter* genus and *H. pylori* species was investigated using polymerase chain reaction by specific primers. **Results:** In total 44% (20/45) and 86.7% (39/45) of samples was positive for *ureC* and *16SrRNA* genes respectively. The frequency of *babA2*, *jhp0917* and *jhp0918* genes in *H. pylori* isolates were 40, 20 and 65% respectively. It seems 19 samples were positive probably non-pylori Helicobacters. **Conclusion:** In the present study we report for the first time the presence of non-*pylori Helicobacter* species and also high frequency of *babA2* and *dupA* genotypes in dental plaque samples.

*Keywords*: *Helicobacter pylori*, Dental plaque, *babA2*, *dupA*.

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## 1. INTRODUCTION

*Helicobacter pylori* (*H. pylori*) is a human gastric pathogen that is present in half of the world's population [1–3]. *H. pylori* associated with gastritis, peptic and duodenal ulcer and risk factor for mucosa-associated lymphoid tissue lymphoma and gastric cancer Gastric cancer (GC) is the major public health issue with a high prevalence in the world [4–6]. The prevalence of *H. pylori* infection is estimated more than 90% in developing country, and 20 to 80% in developed countries [7].

The blood-group antigen-binding adhesion (BabA) is an important virulence factor that in some *H. pylori* strains has been shown to contribute to the pathogenicity of the bacteria [8, 9] Several studies have revealed that the presence of *babA*-positive strains is correlated with developing peptic ulcer disease and gastric adenocarcinoma [8, 10].

Another virulence marker is the duodenal ulcer promoter gene A (DupA). Due to *dupA* is presumably a specific marker for development of duodenal ulcer, the gene was named *dupA* [11, 12]. And also demonstrated it caused protection against gastric atrophy, intestinal metaplasia, and gastric cancer [12, 13].

There are multiple routes of transmission of *H. pylori* infection. Because of identification of *H. pylori* in oral cavity, it has purposed that oral-oral is a major route of *H. pylori* transmission [14].

Dental plaque is the only bacterial biofilm in oral cavity that has been protected from host defense mechanisms and antibiotic therapy [15, 16].

There is a strong possibility of the relationship between *H. pylori* infection by oral-oral transmission and dental plaque in human oral cavity that can provide a micro-environment for colonization of the bacterium [17–19]. Oral-oral transmission can be a cause the gastric infection to return of re-infection after successful eradication of bacterium from stomach and/or treatment failure [18, 20, 21]. However there is a little information about *H. pylori* virulence factors in dental plaques.

Therefore, the purpose of this study was to determine the frequency of *H. pylori babA2* and *dupA* genotypes in dental plaques of patients who are suffering from chronic periodontal diseases from Kermanshah, Iran.

## 2. MATERIALS AND METHODS

### *2.1. Patients and Clinical Specimens*

The patients suffering from chronic periodontal diseases referred to Department of Periodontology, School of Dentistry, Kermanshah University of Medical Sciences. This study was approved by the Ethical Committee of the institution and all patients gave their

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**Table 1.** PCR primers for amplification of *16SrRNA*, *UreC*, *babA2*, *jhp0917* and *jhp0918* sequences

| Target | Primer sequence | Product size, bp | References |
|---|---|---|---|
| *16SrRNA* | GCTATGACGGGTATCC | 422 | (49) |
| | GATTTTACCCCTACACCA | | |
| *ureC* | CATCGCCATCAAAAGCAAAG | 214 | (22) |
| | CAGAGTTTAAGGATCGTGTTAG | | |
| *babA2* | CAATTTAAAGGAGAAAACATGAAAA | 475 | This study |
| | CGTTCAAAGAACAAGTGATGG | | |
| *jhp0917* | ATGAGTTCCATACTAACAGAC | 1198 | This study |
| | TCTCATTAAAATCTTGTTCGC | | |
| *jhp0918* | CAATACCGCTAATACAGATG | 752 | This study |
| | GCACTTGTCTGGCTCTC | | |

informed consent to participate and were signed the consent form. Diabetics, pregnant women, HIV-positive patients, smokers, and patients who used antacids and anti *H. pylori* antibiotics such as amoxicillin, tetracycline, metronidazole and clarithromycin during the last week were excluded. All participants were examined by dentist and dental plaque was collected from the at least 2 teeth surfaces (one anterior and one posterior teeth) by scraping tooth surfaces with sterile curettes.

### *2.2. H. pylori Isolation and PCR*

Dental plaque samples were homogenized with a glass rod and centrifuged in 8000 rpm for 5 minutes. The pellet was subjected to DNA extraction by DNA extraction kit according to the manufacturer's recommendations, (Accuprep Genomic DNA Extraction Kit, Bioneer, South Korea). The extracted DNA had been kept in −20°C before it would be used in PCR reactions. The isolates were definitely identified as *H. pylori* with amplification of the *16SrRNA* and *ureC* specific primers according to previously described procedure [22].

The presence of *dupA* (*jhp0917* and *jhp0918*) and *babA2* genes was investigated using specific primers that designed on the basis of the sequence retrieved from GenBank (accession no. AE001439.1). A pair of primers for *babA2* was designed in order to detect a 10-bp deletion in the signal region of the *babA* gene. The presence of *jhp0917–jhp0918* genes was considered by *dupA* gene of *H. pylori* which are based on design (Table 1).

PCR was carried out in a total volume of 15 μL containing 1X PCR buffer (Sinaclon, Iran), 1.5 mM $MgCl_2$ and 0.6 μM of each primer, 0.2 mM deoxynucleotide, 1U Taq DNA polymerase and 1 μL of template DNA. Also, all reactions were carried out with positive and negative controls in each round of amplification. (*Helicobacter pylori* positive control was kindly received from Alebouye M, Gastroenterology and Liver Diseases Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran).

The PCR reactions were carried out by initial denaturation at 94°C for 5 min and 35 cycles consisting of denaturation, annealing at 94°C for 30 s, annealing at mentioned temperature for 1 min and extension. The final step was extended to 5 min at 72°C. The annealing temperature for each primer was 54, 59, and 54°C for *babA2*, *jhp0918*, and *jhp0917*, respectively. After the amplification. The PCR products were separated on 1% agarose gels (Sinaclon, Iran) in TBE 1× buffer (Tris/borate/EDTA). The DNA bands were stained using ethidium bromide and visualized [23] under UV gel documentation. 422, 214, 475, 1198 and 752 bp bands were considered positive reaction for *16S rRNA*, *ureC*, *babA2*, *jhp0917* and *jhp0918* genes, respectively.

## 3. RESULTS

The study population consisted of 45 dental plaques patients, 35 females (77.8%) and 10 males (22.2%). The mean age of the patients was, 36.82 ± 11.92 and the patients were of 19 and 67 years.

The frequency of *H. pylori* isolation was 44% in the total sample. There was not any significant interaction of age and the dental plaque. In total 39 dental plaque samples (86.7%) was positive for *Helicobacter* genus using *16SrRNA* specific primer and 20 dental plaque samples (44%) was positive for *H. pylori* using *ureC* species-specific primers. It seems 19 samples that were *ureC* negative but positive for 16SrRNA genus specific were probably non-pylori Helicobacters.

The frequency of *babA2*, *jhp0917* and *jhp0918* genes in *H. pylori* isolates were 40, 20 and 65% respectively. The *dupA* gene was detected in only 4 (20%) of our *H. pylori* infections. In 3 samples (15%) were positive both of the *babA2* and *dupA* genes were positive.

## 4. DISCUSSION

*H. pylori* infection is one of the most common bacterial infections in humans, and is known as an important cause of gastritis, peptic ulcer and gastric cancer [8]. Several *H. pylori* virulence genes as *babA2* and *dupA* have been identified that contribute to the risk and severity of these diseases [24, 25]. BabA is one of the outer membrane proteins of *H. pylori*. *BabA1* and *babA2* are encoded by *babA* gene, while *babA1* has a 10-bp deletion in the encoding signal peptide [9]. Therefore, only the *babA2* gene is biologically active and expressed as an adhesive molecule [9, 26]. In studies related to dupA reported that presence of *dupA* was associated with increased mucosal neutrophil infiltration and inversely related to mucosal atrophy and gastric cancer [12, 27]. There is evidence that the *H. pylori* strains reach the stomach by ingestion through the mouth. Oral cavity *H. pylori* was firstly reported in 1989 from dental plaque of a patient with gastric disease [28].

Dental plaque is a bacterial biofilm that helps the *H. pylori* to survive as a potential reservoir in oral cavity [15, 29]. Therefore, the purpose of this study was to determine the frequency of *H. pylori babA2* and *dupA* genotypes in dental plaques of patients with chronic periodontal diseases from Kermanshah, Iran.

The results of some studies are very inconsistent and some studies have reported the presence of *H. pylori* in the oral cavity with vast ranging from 0–100% and it also has confirmed as source of *H. pylori* for gastric re-infection after therapy in patients with positive dental plaque [23, 30] Chaudhry et al., 2011 [31].

While the presence of *H. pylori* in dental plaque was found in some studies, there are also studies that strongly obtained the absence of this bacterium in dental plaques [32, 33]. The reason of different reports could be explained by diversity of the populations studied and/or sampling methods and/or detection of *H. pylori* protocols employed (Song et al., 1999) [34]. For example, Silva et al. reported that the oral cavity is not a reservoir for infection [35]. While in study of Miyabayashi et al. demonstrated that it was the relationship between oral colonization of *H. pylori* infection and gastritis, they also reported that patients Infection with oral *H. pylori* have a significantly elevated gastric re-infection after therapy [36]. More studies emphases a positive association between dental plaque and gastric *H. pylori* [37–39]. Hence it may seems to pay attention to dental plaques as the possible source of reinfection after treatment of gastritis and as a second reservoir of *Helicobacter pylori* colonization be the cause of oral–oral transmission [40]. In study of Brazil, the frequency of the *ureA* and *16SrDNA* genes of *H. pylori* were achieved in 80% of gastric, 30% of saliva and 20% of dental plaque specimens [35].

A few studies have been done about *H. pylori* virulence genes in dental plaque. In some studies, the frequency of *cagA* and *vacA* has been identified. The frequency of *cagA* and *vacA* in different studies were found in 24.1, 48 and 82, 37% dental plaque samples respectively and in the another study they were not able to find *cagA* and *vacA* in dental plaque specimens [38, 41]. The frequency of *babA2* and *dupA* (*jhp0918* and *jhp0917*) in dental plaque samples were 40 and 20% (20 and 40%) respectively. This study showed that there was no statistically significant association between *babA2* and *dupA* genes and dental plaque samples.

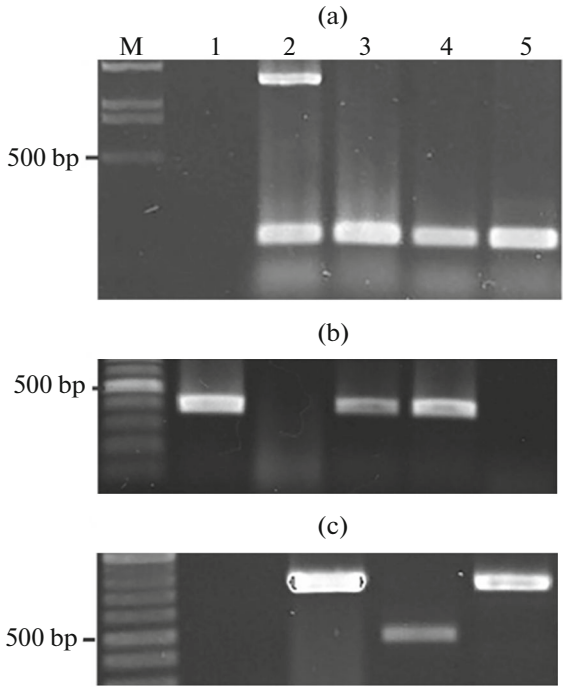


**Fig. 1.** Gel electrophoresis of *Helicobacter* and *H. pylori* genotyping by *16SrRNA*, *ureC*, *babA2*, *jhp0917* and *jhp0918*. (a) M; Lambda DNA/EcoRI+HindIII Marker, (1) negative control, (2) positive isolates for *ureC* and *jhp0917*, lane (3, 4, 5) positive isolates for *ureC*. (b) M; 100 bp marker, (1) positive control, (2) negative control, (3, 4, 5) positive and negative isolates for *16SrRNA*. (c) M; 100 bp marker, (1) negative control, (2) positive isolates for *jhp0918*, (3) positive sample for *babA2*, (4) positive isolates for *jhp0918*.

The wide variation in the prevalence of *H. pylori* in the oral cavity which was found about 5–97% [23, 42–46] but, there was no any report about *16SrRNA Helicobacter* genus positive samples in dental plaque in the previous reports. In the present study 86.7% [39, 45] of dental plaque samples was positive for *Helicobacter* genus by using *16SrRNA*. According to the results which are mentioned above, the other species of *Helicobacter* or non-*pylori Helicobacter* was most frequently found in dental plaque (49%) [19, 39]. There is no previously report about presence of non-pylori *Helicobacter* in human oral cavity [47, 48].

However in this study, there was no statistically significant association between presence of *babA2* and

*dupA* virulence factors in dental plaque was no statistically significant, but the results show a high frequency of the both virulence factors among the samples.

## CONCLUSIONS

In the present study we report for the first time the presence of non-*pylori Helicobacter* species and also high frequency of *babA2* and *dupA* genotypes in dental plaque samples.

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