Clinical Research

Prevalence of *Helicobacter pylori* among patients with different gastrointestinal disorders in Saudi Arabia

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ABSTRAK

Latar belakang: *Helicobacter pylori adalah patogen gastrointestinal yang penting yang berkaitan dengan gastritis, ulkus peptikum, dan peningkatan risiko kanker lambung. Studi ini bertujuan mencari hubungan patogen ini dengan berbagai kelainan gastrointestinal.*

Metode: *150 pasien rawat jalan yang dirujuk ke Saudi Arabian Medical City, Riyadh, Arab Saudi diikutsertakan pada bulan Januari sampai Juni 2015. Setiap pasien menjalani endoskopi dan biopsi diambil spesimennya untuk pemeriksaan urease dan kultur. Koloni yang dicurigai sebagai H. pylori dilakukan identifikasi morfologi, pemeriksaan mikroskopik dan biokimia. Dilakukan juga pemeriksaan PCR untuk mendeteksi gen urease subunit ureA.*

Hasil: *Hasil endoskopi bervariasi dari normal, ulkus gaster, ulkus duodenum, gastritis, dan kanker lambung dengan prevalensi masing-masing 20.7%, 20%, 24%, 33,3%, dan 2%. Pemeriksaan pewarnaan langsung menunjukkan 52% pasien positif H. pylori, sedangkan kultur dan uji cepat menghasilkan prevalensi 71,33%. Lima puluh empat biopsi (36%) menunjukkan urease positif setelah satu jam pada suhu ruang, 39 spesimen (62%) setelah satu jam inkubasi pada 37°C, dan 14 spesimen (71,33%) setelah inkubasi 24 jam. Isolat H. pylori memperlihatkan hasil positif katalase, oksidase, dan urease. Hasil PCR menunjukkan fragment 411-bp, yang sesuai dengan gen urease subunit ureA.*

Kesimpulan: *Prevalensi H. pylori cukup tinggi pada populasi yang diteliti. Terdapat hubungan kuat antara H. pyori dengan ulkus duodenum. Fragmen 411-bp merupakan indikator adanya gen urease subunit ureA.*

ABSTRACT

Background: *Helicobacter pylori* is an important gastrointestinal pathogen associated with gastritis, peptic ulcers, and an increased risk of gastric carcinoma. The present study was carried out to determine the relationship between this organism with different gastrointestinal ailments.

Methods: 150 outpatients referrals to Saudi Arabian Medical City, Riyadh, Kingdom of Saudi Arabia was recruited in January to June 2015. Each patient was subjected to endoscopic examination. Biopsy specimens were taken from the stomach for rapid urease test and culture. Suspected *H. pylori* colonies were subjected to colony morphology identification, microscopical examination and biochemical reactions. The samples were also subjected to PCR to detect ureA subunit of urease gene.

Results: The endoscopic examination of patients revealed normal, gastric ulcer, duodenal ulcer, gastritis, and gastric cancer with a rate of 20.7%, 20%, 24%, 33.3%, and 2%, respectively. Direct smear exam revealed that 52% of patients were *H. pylori* positive while culture and rapid urease test showed a prevalence of 71.33%. Fifty four biopsies (36%) were urease positive after 1 hour at room temperature, 39 (62%) after 1 hour incubation at 37°C and 14 (71.33%) after 24 hours incubation. Isolated *H. pylori* showed that they were catalase, oxidase, and urease positive. PCR results showed 411-bp fragment, which is indicative for the ureA subunit of urease gene.

Conclusion: The prevalence of *H. pylori* infection was high among tested population. Strong association between *H. pylori* and duodenal ulcer was noticed. A 411-bp fragment indicative of the ureA subunit of urease gene was detected in all the tested isolates.

Keywords: duodenal ulcer, gastric ulcer, *H. pylori*, PCR, ureA

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Helicobacter pylori (H. pylori) is one of the most common infections in the world. It presents in 70–90% of the population in developing countries and 35–40% in developed ones.[1] *H. pylori* infection is associated with duodenal ulcer,[2] gastric ulcer and gastric cancer.[3] Approximately, 50% of the normal population across the world harbor *H. pylori*, though only 10–20% of them become symptomatic.[4] There is a high prevalence of *H. pylori* infection in developing countries especially among children. In India, the prevalence of this infection is 22%, 56%, and 87% in the 0–4 years, 5–9 years and in the 10–19 years age group, respectively.[5]

A wide range of laboratory investigations are available for diagnosis of *H. pylori*. The tests are both non-invasive and invasive. Non-invasive tests include, urea breath test, serological immunoglobulin G (IgG), and immunoglobulin M (IgM) detection, saliva and urinary antibody test, and stool antigen test.[6] Culture is also a successful method for diagnosis but it is time-consuming.[7] Rapid urease test is a reliable method for presumptive identification of *H. pylori*[8] and enzyme linked immunosorbant assay (ELISA) is the most common tool because of its speed, low costs, simplicity, and reproducibility.[9]

Polymerase chain reaction (PCR) techniques have also been used for diagnosis of *H. pylori*. It has widespread use in research as it holds great promise in the detection of genetic differences between *H. pylori* strains for epidemiological studies. Vinette et al[10] showed that PCR is the method with potential for greatest sensitivity and specificity in the detection of *H. pylori* specific deoxyribonucleic acid DNA. Huang et al[11] reported a PCR method based on *H. pylori* isocitrate dehydrogenase gene sequence that can rapidly and specifically detect the organism. Furthermore, Brooks et al[7] developed a PCR assay, which is reportedly 100% sensitive and specific for detection of *H. pylori* infection in gastric mucosal biopsy specimens. The study aimed to determine the prevalence of *H. pylori* among 150 outpatients with different gastrointestinal disorders, validate different microbiological diagnostic techniques and detect a subunit of the urease gene (ure*A*) using PCR to confirm the identification of *H. pylori*.

METHODS

Patients

A total of 150 outpatients referrals to Saudi Arabian Medical City, Riyadh, Kingdom of Saudi Arabia with dyspeptic symptoms and clinically indicated for upper gastrointestinal endoscopy were selected. Informed consent mentioning that data from the diagnosis would be used for research purpose was obtained from all the patients. The research protocol was approved by institutional research committee College of Applied Medical Sciences, Shaqra University (53/27844. There were 105 male and 45 female participants with age between 15–75 years. The samples were collected from January to June, 2015. Personal history; name, age, sex, residence, and job were taken from each patient. The main complaints were dyspepsia, nausea, vomiting, abdominal pain and hematemesis. Patients taking antimicrobial drugs and/or bismuth salts within two weeks before endoscopy were excluded from the study.[7]

Endoscopy

Patients were instructed by the gastroenterologist not to eat or drink for at least eight hours before endoscopy procedure. They received oropharyngeal local anesthetic (Xylocain spray 10%). The endoscope and biopsy forceps were cleansed with Savlon and then sterilized by soaking in Cidexin for 10 minutes followed by washing with sterile water. All endoscopic examinations were carried out by using video-endoscopes PENTAX EPM-3500. Three biopsy specimens were taken from antrum region along the greater curvature of stomach, one was biopsy specimen was used for urease test.[12]

Culture

The second biopsy specimen was directly smeared and examined for Gram negative curved bacilli. The third biopsy specimen was homogenized and streaked on fresh brain heart infusion agar supplemented with 10% sterile horse serum and on Colombia blood agar with selective supplement (Dent, Oxoid) and modified Columbia agar (MCUA) slant. The plates were incubated under microaerophilic condition using Gas Generating Kits Campylobacter system BR 0546A (Oxoid, Hampshire, England) at 37°C for 3–5 days. *H. pylori* suspected colonies

were identified by Gram stain and biochemical tests. Biopsies were transported in ice from the endoscopic unit to the microbiological laboratory in 1 ml horse serum supplemented brain heart infusion broth[13] and processed within two hours.[7]

Primary diagnosis of *H. pylori*

The suspected purified colonies were chosen according to the Gram staining and culture's characteristics.

Biochemical tests

Biochemical tests include production of catalase, oxidase, urease, and H_2S, nitrate reduction, growing in 3.5% NaCl, growing with 1% glycine, and growing at different temperatures.

Bacterial DNA extraction and purification

Twenty representative samples from pathological cases were subcultured on selective Colombia blood agar and subjected to DNA extraction and purification. The method is described in the protocol of QIAamp DNA Mini Kit® (QIAGENE Inc., Santo Clarita, Calif.), which contains Proteinase K and animal tissue lysis (ATL), cell lysis (AL), wash buffer 1 (AW1), wash buffer 2 (AW2) buffers. Three bacterial colonies were removed from each culture plate with an inoculating loop and suspended in ATL buffer by vigorous stirring. Twenty µl of Proteinase K was added for each sample and incubated at 56°C in incubator shaker until the sample was completely lysed. The tubes were centrifuged briefly to remove drops from the inside of the lid. Two hundred µl of AL buffer was added, mixed by pulse-vortexing for 15 seconds and incubated at 70°C for 10 minutes. The mixture of each sample was applied to the QIAamp spin column. Tubes were centrifuged at 8,000 rpm for one minute. QIAamp spin column was placed in a clean 1.5 ml microcentrifuge tube, carefully opened and 200 µl of AE buffer was added. The mixture was incubated at room temperature for one minute and centrifuged at 8,000 rpm for one minute.

Amplification of DNA by PCR

The oligonucleotide primers used for the amplification were:

HPU1, (5'-GCC AAT GGT AAA TTA GTT–3') and HPU2, (5'- CTC CTT AAT TGT TTT TAG –3').

This target DNA sequence was used in developing the PCR assay according to Lu et al[14] and He et al[15] followed by agarose gel electrophoresis for PCR products. Gel was photographed using Polaroid Pand Camera with an orange lens filter on T 667 Polaroid film.

Statistical analysis

The obtained results were statistically analyzed using MINITAB statistical software, copyright 1992, release 8, MINITAB INC. Chi-square test was applied to calculate the significance difference between various gastrointestinal disorders groups (e.g. gastric ulcer, duodenal ulcer, gastritis, etc).

RESULTS

A total of 150 outpatients with dyspeptic symptoms and clinically indicated for upper gastrointestinal endoscopy were selected. Among these examined patients, the endoscopic findings revealed that 20.7% (31/150) were normal, 20% (30/150) were suffering from gastric ulcer, 24% (36/150) with duodenal ulcer, 33.3% (50/150) with gastritis and only 2% (3/150) with gastric cancer. Table 2 and Figure 1 showed the distribution of these groups according to endoscopic examination. This result revealed that most prevalent finding was patients with gastritis and the lowest those suffering from gastric cancer as shown in Table 1 and Figure 1.

The culture and biochemical tests showed that 107 (71.33%) out of 150 gastric biopsy specimens were positive for *H. pylori.* The presence of *H. pylori* in gastric biopsy specimens, correlated

Table 1. Demographic and clinical characteristics of patients included in the study (n=150)

Variables	Description
Sex (male/female)	105/45
Age years (range)	(15–75)
Endoscopic finding	n (%)
Normal	31/150 (20.7%)
Gastric ulcer	30/150 (20.0%)
Duodenal ulcer	36/150 (24.0%)
Gastritis	50/150 (33.3%)
Gastric cancer	3/150 (2.0%)
Chief complaints	Dyspepsia, nausea, vomiting, abdominal pain and hematemesis

with the change in the color of the slant MCUA tube from orange to pink that occurred at the same time thus giving an additional evidence for the presence of *H. pylori* in the samples (Figure 2). The colonies of the isolated *H. pylori* were small to middle in size, rounded, and creamy in color. All *H. pylori* isolates were Gram-negative spiral to *coccobacilli* and shared the characteristic catalase, urease, and oxidase production, but differed

Table 2. Frequency of *H. pylori* detection in gastric biopsy specimens by using rapid urease test and culture

Gastric biopsy specimens	*H. pylori* positive n (%)	*H. pylori* negative n (%)
Normal	13/31 (42 %)	18/31 (58%)
Gastric ulcer	19/30 (63%)	11/30 (37%)
Duodenal ulcer	33/36 (92%)	3/36 (8%)
Gastritis	39/50 (78%)	11/50 (22%)
Gastric cancer	3/3 (100%)	0 (0%)
Total	107 (71%)	43 (29%)

Statistical significant at ($p<0.01$)

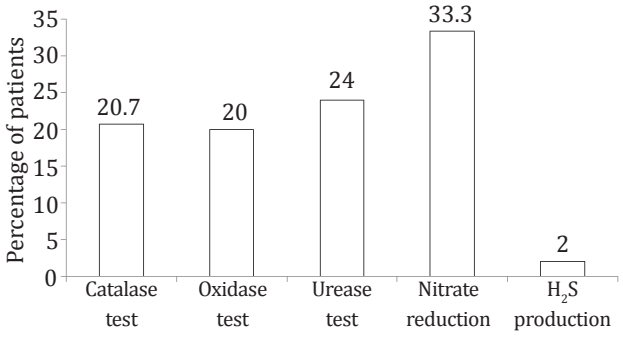

Figure 1. Endoscopic findings in gastric biopsy specimens of studied patients

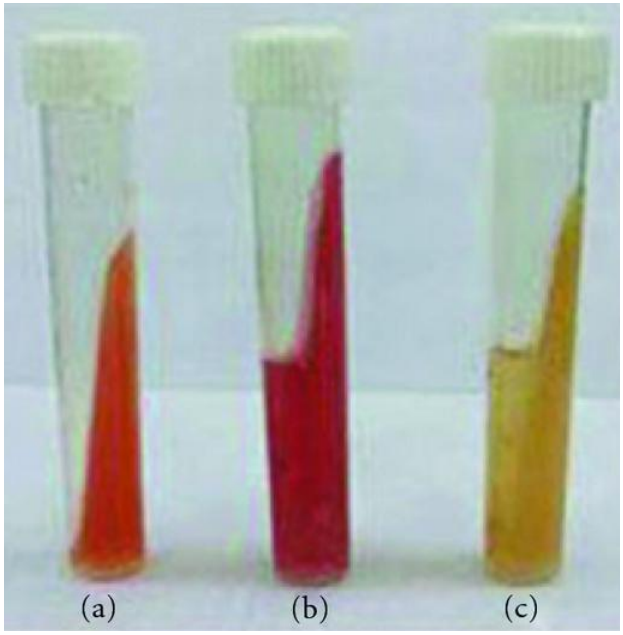

Figure 2. Change in color of slant MCUA tube, a) slant MCUA tube only; b) positive slant MCUA tube culture; c) negative slant MCUA tube culture

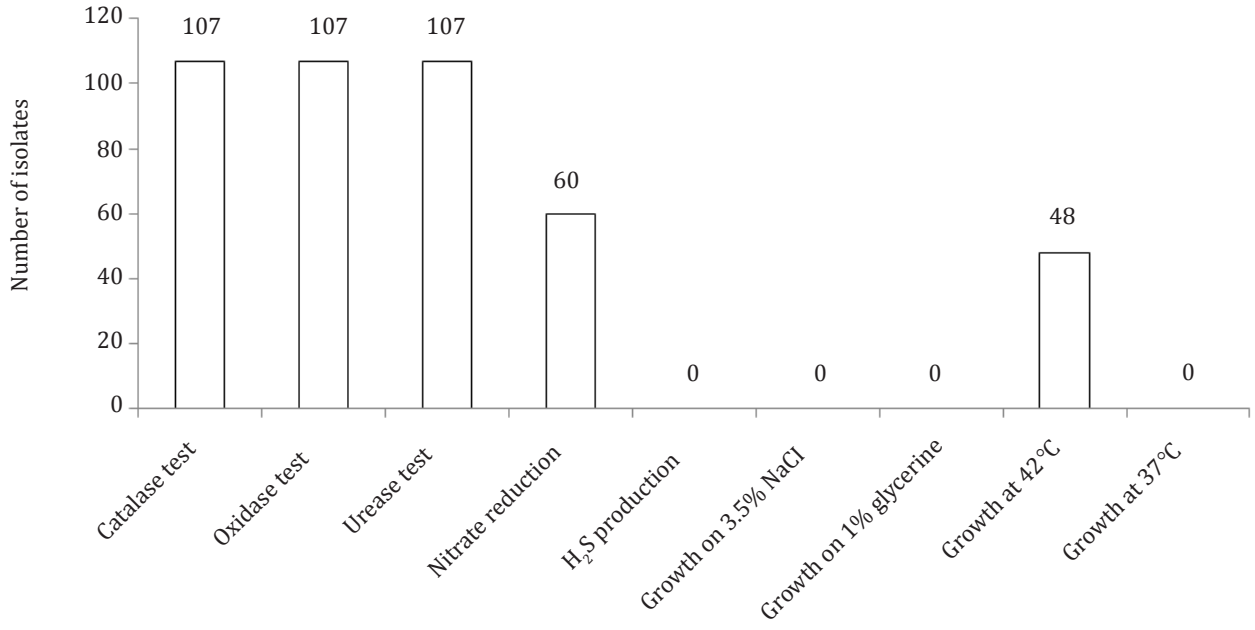

Figure 3. Biochemical tests for identification of *H. pylori* isolates. *107 isolates were positive for *H. pylori* out of 150 isolates

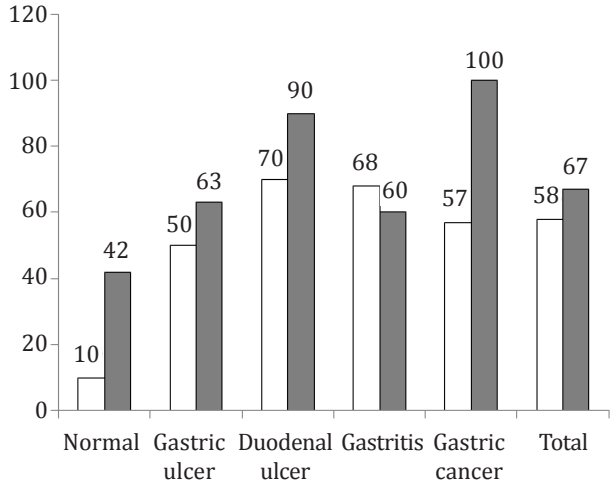

Figure 4. Prevalence of positive *H. pylori* among studied cases

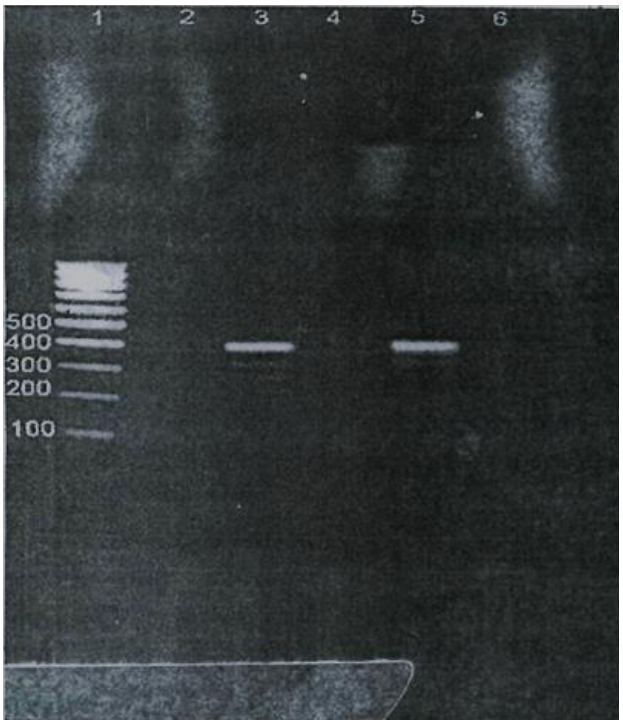

Figure 5. Lane 1 shows molecular weight marker (ladder), lanes 2, 4 and 6 are negative controls and lanes 3 and 5 are PCR positive

slightly with respect to other tests (Figure 3). Collectively, some isolates were being positive in nitrate reduction, some were able to grow at 42°C or both. All isolates were unable to grow on 3.5% NaCl, 1% glycin or at 25°C, respectively.

The culture and urease test revealed that 107 (71.33%) out of 150 gastric biopsy specimens were positive for *H. pylori.* The *H. pylori* was positive in 42% (13/31) of the normal patients, in 63% (19/30), and in 92% (33/36) of patients with gastric and duodenal ulcer, respectively. Seventy eight percent (39/50) of patients with gastritis were *H. pylori* positive. All patients with gastric cancer were *H. pylori* positive. The relation between presence or absence of *H. pylori* and endoscopic findings are shown in Table 2.

Figure 4 shows similarity between culture and rapid urease test results, while highly significant difference between different gastrointestinal groups with Gram stain results.

Samples from 107 isolated *H. pylori* colonies were subjected to PCR. A 41l-bp fragment indicative of the urea subunit of urease gene was obtained with primers specific for this subunit from these isolated strains. This result confirmed that the isolated colonies were *H. pylori*. PCR results are shown in Figure 5. Lane 1 shows molecular weight marker (ladder), lanes 2, 4 and 6 are negative controls and lanes 3 and 5 are PCR positive. All of samples were positive for ureA gene by PCR.

DISCUSSION

The isolation rate of *H. pylori* among dyspeptic patients by culture and rapid urease test was inconsistence with Franzin et al[16] who reported that 75.5% of dyspeptic patients were *H. pylori* positive. On the other hand, our findings were higher than the percentage in the study obtained by Matsukura et al.[12] Studies performed at Ismailia (Egypt), 66% and 68%, respectively of dyspeptic patients were *H. pylori* positive.[17,18] A possible explanation for these differences in isolation rates could be attributed to different sample size and methods used for isolation. The present study revealed that incidence of *H. pylori* among dyspeptic patients with normal endoscopic findings was 42%. This result is similar to the one reported by Suzan[18] and Maii.[17] The incidence of *H pylori* among gastric ulcer patients in this study was 63%. This result was relatively around the spectrum of results reported in different parts of the world, which averaged 70%,[20] while it was significantly lower than that reported by Franzin[16] and Suzan,[18] who recorded 78.6% and 75%, respectively. This could be attributed to gastric reflux or due to the effect of analgesic and anti-inflammatory drugs.

Furthermore, host characteristics and strain viability may play a role in the pathogenesis of peptic ulcer disease.

Among the duodenal ulcer patients, 92% were *H. pylori* positive by culture and urease test (Table 1 and Figure 1). This finding is in agreement with Calvet et al[20] who reported 95% of duodenal ulcer patients with positive *H. pylori*. In a similar study, Li et al[21] recorded a wide range of positive cases (73-100%). On contrary, Laura et al[22] found that duodenal ulcer patients were positive only in 74.3% of studied cases. A strong association between *H. pylori* and duodenal ulcer was observed in our study.

The present work revealed that incidence of *H. pylori* among gastritis patients was 78%. This is close to those reported by Suzan[18] where isolation rate was 67.5%. Moreover, we detected the organism in all patients with gastric cancer. This result is relatively similar to a previous study which reported 98% of gastric cancer patients were *H. pylori* positive.[11]

The results of rapid urease test showed that 71.33% of dyspeptic patients were positive for *H. pylori*. This finding was in close agreement with previous observations of Suzan[18] and Maii[17] who reported 66% and 68%, respectively. On contrary, Oyedeji et al[23] and Brooks et al[7] reported 42.1% and 43.3% positive for *H. pylori* by urease test. These variations in rapid urease test result may be attributed to certain limitations[23] such as the presence of some isogenic urease negative mutants of *H. pylori*[24] or false positive results owing to presence of other urease positive bacteria in the sample.[23] Other possible explanation for conflicting results of rapid urease test could be due to reflux of alkaline bile into the stomach that gave false positive result.[7] Results of rapid urease test were optimized at different environments. Positive results (36%) were obtained at room temperature after one hour. At 37°C after one hour and 24 hours incubation, 62% and 71.33% positive results, respectively were obtained. The results of this study indicate a good correlation between culture and rapid urease test, as all biopsy specimens diagnosed positive by culture were also positive by rapid urease test. Similarly, the previous findings of Suzan[18] and Brooks et al[7] showed complete agreement between culture and rapid urease test results.

We used the ure A gene as a PCR target. A PCR product of the anticipated size (411 bp) was obtained from all of samples. Our result is in agreement with He et al[15] and Vinette et al[10] who reported that ureA gene is conserved and specific to *H. pylori*. On the other hand, Lu et al[14] showed that ureA gene PCR is specific but with low sensitivity (75%), this result was reported when they compared five commonly used primer sets targeting different segments of *H. pylori* genome. They attributed this low sensitivity to sequence polymorphism in this locus. Brooks et al[7] attributed the sensitivity of PCR to the size of the amplified region. They showed that the smaller 294 bp glmM product provides a more accurate result than 411 bp ureA product. These controversial findings in the sensitivity of the target gene may be attributed to different samples as Lu et al[14] and Brooks et al[7] used gastric biopsy samples while in the current study we used *H. pylori* colonies directly. Maii[17] supposed that different PCR protocols, sampling techniques, and different extraction methods may be a possible explanation for these controversial findings.

To conclude, the prevalence of the infection by *H. pylori* is high with strong association between *H. pylori* and duodenal ulcer is noticed. The UreA gene is a strong confirmatory and relevant to *H. pylori* presence in gastroduodenal diseases.

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Conflict of interest
The authors affirm no conflict of interest in this study.

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