in Bangladesh. Data on H. pylori prevalence using bacterial culture is limited in Bangladesh. The aim of the study was to investigate the prevalence of H. pylori in Bangladeshi subjects using bacterial culture. We also determined the prevalence of infection among different age groups and find out the correlation between the prevalence of H. pylori and the related sociodemographic parameters. A cross-sectional study was conducted among randomly selected households from a peri-urban community in Dhaka, Bangladesh to get an idea about H. pylori status in the lower socioeconomic area. Sociodemographic and clinical information and stool specimen for screening H. pylori infection by stool antigen test were collected. Gastric biopsy was collected for culture from those positive in stool antigen test. A total of 287 subjects were screened by stool antigen test, of them, 92.7% were positive for stool antigen test. Of 259 stool antigen positive samples, 59.1% (n = 153) were H. pylori culture positive. Our data suggest that H. pylori infection is significantly associated with age and smoking habit (P value < 0.05). In addition, dyspeptic symptoms are significantly higher in H. pylori culture positive subjects than the H. pylori culture negative subjects. Endoscopic examination suggests that the gastroesophageal pathologies are significantly associated (P value < 0.05) and duodenal pathologies are moderately associated (P value 0.059) with H. pylori infection. So, H. pylori culture test is auspicious the significant colonization that might lead to pathological outcomes.

Key words: H. pylori; Bangladesh; Prevalence; Gastrointestinal disorders

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INTRODUCTION

Helicobacter pylori inhabit at least 50% of the world’s population, the
prevalence of *H. pylori* infection appears to be higher in developing countries as compared to industrially developed countries and the prevalence vary between populations and between groups within the same population[1,2]. The prevalence in Asia and Africa varies from 54.7% -79.1%, in North and Latin America the prevalence is 37.1% and 63.4% and in Europe, the prevalence is typically 47.0%[3].

*H. pylori* infection may be acquired at any age but once acquired, the infection persists for years and often for the lifetime. Although the route(s) of transmission of *H. pylori* infection remains dubious, the gastro-oral, oral-oral and fecal-oral routes are being speculated[4]. Recently, intrafamilial transmission of *H. pylori*, predominantly from mother to child has been reported[5,6]. In both developing and developed countries, a high prevalence of *H. pylori* is seemingly related to poor socioeconomic conditions, such as overcrowded housing, low income, and the use of a stove for heating or cooking[7]. A variety of clinical outcomes is associated with *H. pylori* infection has been reported including chronic gastritis, peptic and duodenal ulcer, gastric cancer, lymphomas, gastroesophageal reflux disease and adenocarcinoma of the gastric cardia[8,9].

In a study conducted in 1995, 92% of the Bangladeshi population was found to be *H. pylori* positive by ELISA[10,11]. In the same time, 67% of the children of a lower socioeconomic area was found to be *H. pylori* positive detected by urea breath test[12]. It was found that re-infection of *H. pylori* was 5.02% per year in the *H. pylori* eradicated patients using urea breath test[13]. 16s rRNA-based *H. pylori* specific PCR study showed that 67% of the dyspeptic patients in Chittagong were *H. pylori* positive[14]. However, prevalence data in the lower socioeconomic area of Bangladesh was not available for a long time and existing prevalence studies were not confirmed by culture test, which is considered as gold standard for *H. pylori* detection.

The objectives of this study were to estimate the prevalence of *H. pylori* infection in Bangladeshi subjects in the lower socioeconomic area by *H. pylori* stool antigen and culture. We also analyzed the relationship of *H. pylori* prevalence with different sociodemographic parameters, dyspeptic symptoms as well as endoscopic findings.

**MATERIALS AND METHODS**

**Study population**

A cross-sectional study was conducted at Nandipara (a peri-urban community), 10 km from Dhaka, Bangladesh between July 2005 and November 2007. Since 1985, Clinical Services Divisions (CSD), ICDDR, B, maintained a household listing of Nandipara and a database of 3000 population and used to provide routine clinical services through outpatient clinic. We used the database and infrastructure of CSD, ICDDR, B, for selection and enrollment of patients during the study period. One hundred and twelve families from Nandipara community were randomly selected for the study; the selected families including the family head were (1) invited to visit the outpatient clinics at Nandipara; (2) approached to participate in the study; and (3) screened for inclusion/exclusion criteria (exclusion criteria includes concomitant disease; regular NSAID or corticosteroid use; treatment with antibiotics or proton-pump inhibitors for any household members in last 6 weeks; and pregnancy). Of the 112 families approached, fifty-five families (312 family members) were eligible for enrollment and agreed to provide a fecal sample for stool antigen testing. The sample collection from this population to culture *H. pylori* has been described in a flow diagram (Figure 1). The study was approved by Ethical review Committee (ERC) of icddr,b (Approval # 2005-010). Informed consent were obtained from the participants (individual consent for all members above the age of 18 and parental consent for children below 18 were obtained) for enrollment. Families were compensated for wage lose and transportation[15].

**Questionnaire**

Subjects were interviewed by using a pre-structured standard questionnaire. Socio-demographic information (e.g. age, Gender, occupation, individual education level, size of family, approximate monthly family income, marital status, self-reported socioeconomic group, individual hygiene and smoking habits) medical history (hyperacidity, heartburn, acid eruption, epigastric and abdominal pain etc) and medication history (NSAID and proton pump inhibitors in last two months) for each family member were also collected.

**Stool for antigen test**

Morning stool specimen from each family member was collected and examined for *H. pylori* stool antigen using a commercial enzyme immunoassay (FemtoLab *H. pylori* Cnx, DakoCytomation Ltd, United Kingdom) as recommended by the manufacturers (the sensitivity and specificity of the test were 78.9% and 87.0%, respectively)[16]. All *H. pylori* stool antigen-positive subjects (irrespective of participation to upper GI endoscopy) were offered treatment with standard triple therapy.

**Endoscopic examination**

All stool antigen test positive members of the enrolled household were invited for upper gastrointestinal endoscopy and/or gastric juice collection at ICDDR,B clinical research unit. Information on clinical symptoms related to gastric pathologies were collected by physician before endoscopy (epigastric pain, acid eruption, heartburn, anorexia, nausea, vomiting etc). Upper gastrointestinal endoscopy was conducted in a standard fashion with visualization of the esophagus, stomach, and duodenum using a short-acting sedative (10 mg lidocaine) and local anesthetic spray. Endoscopy examination was done by trained endoscopist using a sterile endoscope for each participant (GIF XQ 30, Olympus Optical Company, Japan). Inflammation of the esophagus, stomach, and duodenum were referred to as esophagitis, gastritis and duodenitis respectively. The esophageal mucosal erosion caused by the reflux of gastric component back to the throat through the esophagus was referred to as reflux esophagitis (Clinically this is known as Gastroesophageal Reflux Disease (GERD)). If the stomach's lining was eroded, gets swollen or inflammed lesions was denoted as erosive gastritis. The condition in duodenum with inflamed duodenal wall becomes eroded and open sores formed was denoted as erosive duodenitis. Antral gastritis was denoted while inflammation occurred in the lower portion of the stomach (Antrum). Ulcer formation in stomach and duodenum referred to as ulcerated.

**Culture of Biopsy samples and Gastric juice**

Two biopsies were taken (antrum, corpus) using a sterilized endoscope in each adult patients. In younger children (Age ≤ 12 years), gastric juice samples were aspirated through a sterile nasogastric tube. The biopsy samples were placed in separate 1 ml Stuart transport media and transported on ice to the laboratory and processed within three hours of collection. Biopsy samples were vortexed vigorously for 5 min and the pH of gastric juice was adjusted to 7.4 using 1M Tris HCL. Both biopsy and gastric juice samples were plated on brain heart infusion agar (Oxoid, Ltd, Basingstoke, Hampshire, United Kingdom) supplemented with 7% sheep blood, 0.4% IsoVitaleX, H. pylori Denti supplement
(Oxoid). Plates were incubated at 37°C in an atmosphere of 10% CO2, 85% N2 and 5% O2, for 3 to 6 days. H. pylori colonies were identified based on their typical morphology, characteristic appearance on Gram staining and the production of oxidase, catalase, and urease according the procedures described elsewhere\(^{17}\). A pure culture of pooled isolates was stored at -80°C in 0.5 mL of brucella broth with 15% glycerol. The method of determining the mixed infection from H. pylori culture was described previously\(^{19}\).

**Statistical analysis**

The data were recorded and analyzed by using SPSS for Windows (Version 17, Chicago, IL, USA). Data were analyzed by chi-square (χ²) test. The level of statistical significance was set at 0.05, and all tests were two-tailed. Odds ratio (OR) and 95% confidence intervals (CI) were calculated by unconditional logistic regression model to analyze the risk factors (H. pylori culture positive and smoking) and test the different dyspeptic symptoms as outcomes. We also performed logistic regression model to find the combined effect of H. pylori and smoking in case of dyspeptic symptoms.

**RESULTS**

Among 112 eligible households invited to participate the study 55 households agreed to participate the study. Stool specimen of 287 subjects from 55 households was screened by stool antigen test and of them, 266 (92.7%) was positive for H. pylori. All 266 H. pylori positive subjects were invited for upper gastrointestinal endoscopy and of them, 259 agreed to provide biopsy and/or gastric juice specimen (in children ≤ 12 year n = 49) for culture. Of these 259 subjects, 153 (59.1%) were culture positive for H. pylori (Figure 1).

The Sociodemographic characteristics of the enrolled patients are presented in Table 1. Approximately half of the patients were male, 46.7% were children, and 53.3% were adult. Half of the patients had primary education and 15.3% of the patients reported smoking habit. 44.3% (77/174) were infected with mixed strains of H. pylori, 40.2% (104/259) reported dyspeptic symptoms and most of the patients 93.6% (150/160) had one or more gastrointestinal pathology.

The prevalence of H. pylori infection as determined by stool antigen test and by the culture of biopsy specimen among enrolled subjects (stratified by age, gender, income, education and smoking habit) is shown in Table 2. The stool antigen test detected H. pylori antigen in 92.7% of subjects. The prevalence of HP was 91.9% in subjects below 15 years of age. Such rate continued to be maintained with increasing age (Table 2). Among the subjects who provided biopsy samples, 59.1% were H. pylori culture positive. A significant relationship between the age and H. pylori infection (P value < 0.05) was observed (Table 2). H. pylori infection was significantly higher in patients with smoking habit compared to non-smokers (82.5%; 33/40 versus 54.8%; 120/219) (P value < 0.05). However, no significant difference in prevalence was observed when compared to gender, income, and education (Table 2). We also checked the prevalence of mixed infection in different age groups, smokers and genders. The result showed a significant difference (P value < 0.05) where the single and mixed infection was calculated to be increased with age and smoking habit but not significantly associated with gender (Table S3).

Among 259 subjects those agreed for upper GI endoscopy 160 had one or more pathological conditions as determined during endoscopic examination. Of them, 77% (123/160) were H. pylori culture positive. Among these 160 subjects, 75 (46.9%) have esophageal abnormalities, 149 (93.1%) have gastric abnormalities and 60 (37.5%) have duodenal abnormalities (Table 1). Patients with esophageal and gastric pathology had significantly higher H. pylori culture positive (86.7% vs 68.2%; P value 0.02 and 78.5% vs 54.6%; P value 0.001). The esophageal, gastric and duodenal pathologies of the patients are presented in Table 3. We also examined the relationship between gastrointestinal pathology and stool antigen positivity and no correlation was observed (Table S1).

Binary logistic regression model was used to find the odds ratio for dyspeptic symptoms, where the dyspeptic symptoms were independent variable and H. pylori culture positivity as well as smoking habit was dependent variable. H. pylori culture positive subjects have 2.53 times more probability of epigastric and abdominal pain (OR 2.53; 95% CI 1.28-5.01), 2.53 times more probability of heartburn (OR 2.53; 95% CI 1.43-4.48), and 4.03 times more probability of nausea (OR 4.03; 95% CI 0.87-18.56) (P value < 0.05) (Table 4). H. pylori culture positive subjects have 3.46 times higher probability of dyspeptic symptoms (51.6% vs 23.6%; OR 3.46; 95% CI 1.99-5.99). These dyspeptic symptoms are also significantly higher in smokers compared to non-smokers (P value < 0.05) (Table S3). Whereas, Smoking habit was significantly associated with only the duodenal pathologies but not with esophageal and gastric pathologies (Table S4).

**DISCUSSION**

The prevalence of H. pylori infection varies worldwide, with major risk factors of the poor living condition and infected family members, have great public health implications\(^{19}\). Nandipara is a periurban area with lower socioeconomic condition. Study of H. pylori in this lower socio-economic area adjacent to Dhaka city has an important implication as it might give a clear picture not only the other slum/ periurban area of Dhaka city but also assume H. pylori condition of entire Bangladesh. H. pylori culture showed a high prevalence of Helicobacter pylori in the studied population. Approximately 93% of the population was H. pylori positive by stool antigen test and among those, 59.1% were H. pylori culture positive. High prevalence of H. pylori infection was found in every group based on age, gender, income, education and smoking habit while analyzed with stool
In recent years, risk factors for *H. pylori* infection have been investigated around the world. *H. pylori* infection was thought to be related to socioeconomic status, however, the results from different groups have been contradictory[20]. Hence, the risk factors for *H. pylori* infection are still unclear[21]. Malcolm et al[22] reported that the *H. pylori* infection were associated with age, gender, and socioeconomic conditions. However, our findings suggested that there was no association between the *H. pylori* infection and gender but related to age. Previous studies showed that the prevalence of *H. pylori* infection increased with age, and the prevalence was lower in subjects younger than 20 years old[23]. Our data suggest a similar prevalence of *H. pylori* in different age groups in case of stool antigen report by ELISA, while the prevalence of *H. pylori* infection was increased with the age when considered the culture report. So, the colonization of *H. pylori* is a very slow process and emerge with the increased age. As age is a factor for having *H. pylori* culture positive, we compare the previous mixed infection data in different age groups, gender, and smokers[13]. The data also showed a significant association of *H. pylori* mixed infection with age and smoking habit but not with gender.

The prevalence of *H. pylori* in children varies in the developed and developing countries and in different age groups[24]. In the present study, 92.5% children were found to be *H. pylori* stool antigen positive and 38.0% of them were *H. pylori* culture positive (Table 1). Among the 49 gastric juice samples from younger children (age < 12), 24 (48.97%) were culture positive. This result is alarming as they are culture positive at their early age and are at high risk of gastrointestinal disorders. This is to be noted that due to technical difficulties culture positivity from gastric juice might be low, so, even higher prevalence might exist. The reason for this high prevalence

### Table 1 The characteristics of study sample with their demographic information. (*n* = 287, *n* = 259 for culture test, *n* = 160 for gastrointestinal disorders).  

| Age                | Child (Age < 18) | Adult (Age > 18) | Total |
|--------------------|------------------|------------------|-------|
| < 15               | 112 (100.0%)     | 112 (39.0%)      |       |
| 16-25              | 22 (30.6%)       | 50 (69.4%)       | 72 (25.1%) |
| 26-35              | 24 (100.0%)      | 24 (8.4%)        |       |
| 36-45              | 49 (100.0%)      | 49 (17.1%)       |       |
| > 46               | 30 (100.0%)      | 30 (10.4%)       |       |
|                   | 134 (46.7%)      | 153 (53.3%)      | 287   |

| Gender             |                   |                   |       |
|--------------------|-------------------|-------------------|-------|
| Male               | 71 (47.7%)        | 78 (52.3%)        | 149 (51.9%) |
| Female             | 63 (45.67%)       | 75 (54.3%)        | 138 (48.1%) |

| Income             |                   |                   |       |
|--------------------|-------------------|-------------------|-------|
| Lower (< Tk5000)   | 133 (48.9%)       | 139 (51.1%)       | 272 (94.8%) |
| Higher (> Tk5000)  | 1 (6.7%)          | 14 (93.3%)        | 15 (5.2%) |

| Education          |                   |                   |       |
|--------------------|-------------------|-------------------|-------|
| Illiterate         | 41 (32.8%)        | 84 (67.2%)        | 125 (43.6%) |
| Primary education  | 92 (57.1%)        | 69 (42.8%)        | 161 (56.1%) |
| Secondary education| 1 (100.0%)        | 0 (0.0%)          | 1 (0.3%) |

| Smoking habit      |                   |                   |       |
|--------------------|-------------------|-------------------|-------|
| Smoker             | 0 (0.0%)          | 44 (100.0%)       | 44 (15.3%) |
| Non smoker         | 134 (55.1%)       | 109 (44.9%)       | 243 (84.7%) |

| *H. pylori* stool antigen test |                   |                   |       |
|--------------------------------|-------------------|-------------------|-------|
| Positive                       | 124 (46.6%)       | 142 (53.4%)       | 266 (92.7%) |
| Negative                       | 10 (47.6%)        | 11 (52.4%)        | 21 (7.3%) |

| *H. pylori* culture           |                   |                   |       |
|--------------------------------|-------------------|-------------------|-------|
| positive                       | 46 (50.1%)        | 107 (69.9%)       | 153 (59.1%) |
| Negative                       | 75 (70.8%)        | 31 (29.2%)        | 106 (40.9%) |

| Types of infection            |                   |                   |       |
|--------------------------------|-------------------|-------------------|-------|
| No infection                  | 33 (71.7%)        | 13 (28.3%)        | 46 (26.4%) |
| Single infection              | 13 (25.5%)        | 38 (74.5%)        | 51 (29.3%) |
| Mixed infection               | 25 (32.5%)        | 32 (67.5%)        | 77 (43.3%) |

| Gastrointestinal disorder     |                   |                   |       |
|--------------------------------|-------------------|-------------------|-------|
| Normal                        | 7 (70.0%)         | 5 (30.0%)         | 10 (6.3%) |
| Pathological condition        | 25 (16.7%)        | 125 (83.3%)       | 150 (93.7%) |
| Esophagus                     | 2 (2.7%)          | 73 (97.3%)        | 75 (46.9%) |
| Stomach                       | 25 (16.8%)        | 124 (83.2%)       | 149 (93.1%) |
| Duodenum                      | 3 (5.0%)          | 57 (95.0%)        | 60 (37.5%) |

| Dyspeptic symptoms            |                   |                   |       |
|--------------------------------|-------------------|-------------------|-------|
| Normal                        | 112 (72.3%)       | 43 (27.7%)        | 155 (59.8%) |
| - Dyspepsia                   | 9 (8.7%)          | 95 (91.3%)        | 104 (40.2%) |
| Pain                          | 5 (8.6%)          | 53 (91.4%)        | 58 (22.4%) |
| Acid eructation               | 1 (5.6%)          | 17 (94.4%)        | 18 (6.9%) |
| Heartburn                     | 7 (7.9%)          | 92 (92.1%)        | 99 (34.4%) |
| Anorexia                      | 0 (0.0%)          | 20 (100.0%)       | 20 (7.7%) |
| Nausea                        | 0 (0.0%)          | 13 (100.0%)       | 13 (5.0%) |
| Vomiting                      | 0 (0.0%)          | 14 (100.0%)       | 14 (5.4%) |

* The subject show any of the single or multiple dyspeptic symptoms.
in children might be the poor hygienic condition as well as higher intra-familial transmission from mother to child in that lower socioeconomic area[18,19].

Multivariate logistic regression model analysis revealed that annual family income was an important risk factor for H. pylori infection[20]. Our data is not showing any significant difference in the monthly income and H. pylori culture positive. There is a similar prevalence of H. pylori infection in both of the lower (57.4%) and comparatively higher (64.3%) income group (Table 1). In that low socioeconomic area, we found a small number of families who have a high income in respect with that area. So this may be limiting factor in this case. It is generally considered that risk factors for H. pylori infection include sharing a bed with others in childhood, more siblings, more family members, and lower education status[21]. We found a significant correlation between number of family members and H. pylori infection (P value 0.002, data not presented). In the families with more family members, there is a high rate of infection with H. pylori.

There was a general trend in which higher education status was associated with a lower prevalence of infection, and it was confirmed by multivariate logistic regression analysis[22]. However, we did not find any significant difference between H. pylori infection and educational status.

Based on H. pylori culture data, we found a significant relationship between smoking and H. pylori infection (Table 2). So, smoking might be a risk factor for the H. pylori infection in Bangladesh. Several studies have reported the relationship between smoking and H. pylori infection, but none of these studies showed a positive association[22-23]. Interestingly, all of the studies detected H. pylori from serum sample by ELISA, but not used culture as the gold standard for H. pylori detection[22]. So, it might be possible that H. pylori find a suitable environment to colonize in the stomach of smokers leading to culture positive. It has been reported that H. pylori infection, smoking, and heavy drinking increase the risk of gastric cancer[23]. The present analyses showed that smoking was associated with dyspeptic symptoms and duodenal pathology, however not related to gastro-esophageal pathology (Table S4 and S6). Surprisingly the outcome was supported by a previous study reporting smoking and H. pylori synergistically contribute to duodenal ulceration[24].

The subjects with gastrointestinal signs have a high prevalence of H. pylori. We found a significant difference of H. pylori infection between subjects with gastroesophageal and gastroduodenal abnormalities and subjects with no abnormalities. The association between H. pylori infection and chronic gastritis was recognized early[30]. A crucial role of the infection in peptic ulcer disease has been firmly established[30] and accumulating data have also supported an association between H. pylori infection and gastric cancer[31]. We found a relationship between esophageal and gastroduodenal abnormalities and H. pylori infection, providing evidence for the probable involvement of H. pylori in the progression of gastrointestinal abnormalities. Especially, 100% of the subjects with duodenal ulcer and reflux esophagitis as well as 97.6% subjects with erosive gastritis were H. pylori culture positive. Our data also suggest H. pylori may be a risk factor for dyspeptic symptoms.

### Table 2 Prevalence of H. pylori infection among 287 subjects enrolled in the study. Subjects were screened for H. pylori by stool antigen test. Biopsy specimen from stool antigen test positive subjects were used for culture. H. pylori prevalence was compared among different age, gender, income, education, smoking behavior and gastric symptoms.

| Variable       | HP stool antigen positive (n = 287) (Percentage) | HP culture positive (n = 259) (Percentage) | P-value for HP culture |
|----------------|-----------------------------------------------|------------------------------------------|------------------------|
| Age            |                                               |                                          |                        |
| <15            | 103 (91.9)                                    | 35 (35.0)                                | <0.05                  |
| 16-25          | 68 (94.4)                                     | 48 (71.6)                                |                        |
| 26-35          | 24 (100.0)                                    | 17 (73.9)                                |                        |
| 36-45          | 45 (91.8)                                     | 33 (73.3)                                |                        |
| >46            | 26 (86.7)                                     | 20 (83.3)                                |                        |
| Gender         |                                               |                                          |                        |
| Male           | 142 (95.3)                                    | 80 (57.6)                                | 0.342                  |
| Female         | 124 (89.8)                                    | 73 (60.8)                                |                        |
| Income         |                                               |                                          |                        |
| Lower (< Tk5000) | 251 (92.3)                                    | 144 (57.4)                               | 0.438                  |
| Higher (> Tk5000) | 15 (100.0)                                    | 9 (64.3)                                 |                        |
| Education      |                                               |                                          |                        |
| Illiterate     | 117 (93.6)                                    | 67 (58.3)                                | 0.463                  |
| Primary education | 148 (91.9)                                  | 86 (60.1)                                |                        |
| Secondary education | 1 (100.0)                                  | 0 (0.0)                                  |                        |
| Smoking Habit  |                                               |                                          |                        |
| Smoker         | 41 (93.2)                                     | 33 (82.5)                                | <0.05                  |
| Non-smoker     | 225 (92.6)                                    | 120 (54.8)                               |                        |
| Total          | 266 (92.68%)                                  | 153 (59.07%)                             |                        |

### Table 3 Prevalence of esophageal, gastric and duodenal pathological conditions as observed during endoscopic examination and prevalence of H. pylori (n = 160).

| Variable       | Participant (%) | HP positive (%) | P-value |
|----------------|-----------------|-----------------|---------|
| **Esophagus**  |                 |                 |         |
| Normal         | 85 (53.1)       | 58 (68.2)       | 0.02    |
| Esophagitis    | 73 (45.6)       | 63 (86.3)       |         |
| Reflux esophagitis | 2 (1.3)       | 2 (100.0)       |         |
| **Stomach**    |                 |                 |         |
| Normal         | 11 (6.9)        | 6 (54.6)        | 0.001   |
| Gastritis      | 94 (58.8)       | 68 (72.3)       |         |
| Erosive gastritis (EG)* | 42 (25.6)   | 41 (97.6)       |         |
| Antral Gastritis | 13 (8.1)       | 8 (61.5)        |         |
| **Duodenum**   |                 |                 |         |
| Normal         | 100 (62.5)      | 72 (72.0)       | 0.059   |
| Erosive duodenitis | 47 (29.38)  | 38 (80.9)       |         |
| Ulcerated      | 13 (8.1)        | 13 (100.0)      |         |

*One sample was from a patient having erosive gastritis along with ulcer and gastric polyp. The subject was H. pylori culture positive.

### Table 4 The dyspeptic symptoms in H. pylori culture positive subjects compared with H. pylori culture negative subjects.

| Symptoms                  | H. pylori culture positive (n = 153) | H. pylori culture negative (n = 106) | P       | P*       | Odds ratio* | 95% confidence interval* |
|---------------------------|-------------------------------------|-------------------------------------|---------|---------|------------|--------------------------|
| Pain                      | 40 (26.1%)                          | 13 (12.3%)                          | 0.004   | 0.008   | 2.53       | 1.28-5.01                |
| Acid eructation           | 10 (6.54%)                          | 6 (5.66%)                           | 0.496   | 0.774   | 1.17       | 0.41-3.31                |
| Heartburn                 | 61 (39.9%)                          | 22 (20.8%)                          | 0.001   | 0.001   | 2.53       | 1.43-4.48                |
| Anorexia                  | 18 (11.8%)                          | 0 (0.00%)                           | <0.001  | 0.996   | **         | **                      |
| Nausea                    | 11 (7.2%)                           | 2 (1.9%)                            | 0.046   | 0.074   | 4.03       | 0.87-18.56               |
| Vomiting                  | 11 (7.2%)                           | 3 (2.8%)                            | 0.104   | 0.141   | 2.66       | 0.72-9.77                |
| Dyspepsia                 | 79 (51.6%)                          | 25 (23.6%)                          | <0.001  | <0.001  | 3.46       | 1.99-5.99                |

* values found from logistic regression analyses. ** irrelevant data may be due to absence of signs. » The subject show any of the single or multiple dyspeptic symptoms.
Table S1 Relationship between Stool antigen positivity and gastrointestinal symptoms. Most of the subjects with normal endoscopic examination were ELISA positive similar to the symptomatic subjects. Very low number of subjects were stool antigen negative.

| Stool antigen positivity | Gastro-duodenal abnormalities | P value |
|--------------------------|-------------------------------|---------|
| ELISA positive (%)       | Normal                        | 0.333   |
| ELISA negative (%)       | Esophagitis                   | 0.001   |
|                         | Reflux esophagitis            | 0.001   |

Stomach

- Normal: 11 (90.9, 11.1), 0.289
- Gastritis: 94 (98.9, 0.1), 0.041
- Erosive gastritis (EG)*: 42 (97.6, 1.2), 0.241
- Antral gastritis: 14 (100.0, 0.0), 0.001

Duodenum

- Normal: 101 (97.0, 3.0), 0.4
- Erosive duodenitis: 47 (100.0, 0.0), 0.001
- Ulcerated: 13 (100.0, 0.0), 0.001

*one sample was from a patient having erosive gastritis along with ulcer and gastric polyp. The subject was H. pylori ELISA positive.

Table S2 The prevalence of Mixed H. pylori infection in different age groups.

| Age      | Single infection | Mixed infection | H. pylori culture negative | P-value |
|----------|------------------|-----------------|---------------------------|---------|
| < 15     | 10 (12.54%)      | 19 (22.67%)     | 2 (22.22%)                | 0.215   |
| 16-25    | 35 (31.25%)      | 25 (32.08%)     | 5 (6.25%)                 | 0.001   |
| 26-35    | 6 (35.29%)       | 18 (66.7%)      | 4 (23.53%)                | 0.001   |
| 36-45    | 12 (37.50%)      | 16 (50.0%)      | 6 (12.50%)                | 0.011   |
| > 46     | 8 (42.11%)       | 10 (52.63%)     | 1 (5.26%)                 | 0.001   |

Gender

- Male: 33 (35.86%), 36 (39.13), 23 (25.00%), 0.124
- Female: 18 (21.95%), 41 (50.00%), 23 (28.05%), 0.018

Smoking

- Yes: 13 (43.33%), 15 (50.00%), 2 (6.67%), 0.018
- No: 38 (26.39%), 62 (43.06%), 44 (30.56%), 0.001

Our findings suggested that H. pylori culture data was associated with gastrointestinal pathologies (Table 3) but ELISA data was not associated (Table S2). ELISA is a sensitive technique, whereas culture is a confirmatory technique. So, the persons who are positive in ELISA but negative in culture test, might not be significantly colonized by H. pylori. This might be a cause that dyspeptic and gastrointestinal symptoms developed more in a culture positive subjects than the culture negative subjects (though ELISA positive).

In conclusion, the prevalence of H. pylori infection is high in that lower socioeconomic group in Bangladesh. As a developing country, Bangladesh is at high risk for Gastric diseases, such as gastric and duodenal ulcer and Gastric cancer. The prevalence of H. pylori infection was related to age, the size of family, smoking habit, but was not related to gender, education and family income. All the gastroesophageal and gastroduodenal abnormalities are majorly related with H. pylori culture test emphasizing the culture test as a marker for those symptoms while many asymptomatic subjects might be ELISA positive. Our suggestion will be to confirm H. pylori colonization by culture test of the subjects with gastrointestinal symptoms mostly ulcers and cancer. This study represents only the status of lower socioeconomic areas, so detailed studies will be required to understand the H. pylori status and their risk factors in nationwide to emphasize on H. pylori eradication strategy in Bangladesh.

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