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Major Article

Risk factors of *Helicobacter pylori* infection in an urban community in Northeast Brazil and the relationship between the infection and gastric diseases

***Irigrácin Lima Diniz Basílio*[1],[2], *Maria de Fátima Celino Catão*[1], *Janilson Dantas de Sousa Carvalho*[1], *Francisco Paulo Freire-Neto*[3],[4],[5], *Leonardo Capristano Ferreira*[3],[4],[5] *and Selma Maria Bezerra Jerônimo*[2],[3],[4],[5]**

[1]. Universidade Federal de Campina Grande, Campina Grande, PB, Brasil.
[2]. Programa de Pós-Graduação em Saúde, Centro de Ciências da Saúde, Universidade Federal do Rio Grande do Norte, Natal, RN, Brasil.
[3]. Departamento of Bioquímica, Centro de Biociências, Universidade Federal do Rio Grande do Norte, Natal, RN, Brasil.
[4]. Instituto de Medicina Tropical Rio Grande do Norte, Universidade Federal do Rio Grande do Norte, Natal, RN, Brasil.
[5]. Instituto Nacional de Ciência e Tecnologia de Doenças Tropicais, Natal, RN, Brasil.

Abstract

Introduction: *Helicobacter pylori*, a water contaminant, is the primary pathogenic agent associated with gastric diseases in humans. Exposure to *H. pylori* is more likely higher in developing countries. This study aimed to evaluate the risk factors associated with *H. pylori* infection in patients undergoing endoscopy to validate the cause of dyspeptic symptoms in an urban population in northeast Brazil and to compare the urease test and polymerase chain reaction assay results with the histopathological findings. **Methods:** We evaluated 200 of 759 individuals with dyspeptic complaints from Campina Grande, State of Paraiba, northeast Brazil. Patients underwent endoscopy, followed by gastric biopsies. Logistic regression analysis was performed to adjust for confounders and to determine significant risk factors of dyspeptic disorders. **Results:** Women accounted for 72.5% (145/200) of the participants. Approximately 59.8% (120/200) of the samples tested positive for *H. pylori* based on histological examinations. The specificity of polymerase chain reaction assay was higher than that of the urease test (77% vs. 64%, p=0.034). City drinking water [odds ratio (OR): 2.6; 95% confidence interval (CI): 1.3-5.21; p=0.004] and smoking (OR: 4.0; 95% CI: 1.13-14.5; p=0.031) were the risk factors of *H. pylori* infection. Belching was the most common symptom associated with *H. pylori* infection (p=0.05). **Conclusions:** The increased risk of *H. pylori* infection associated with non-treated water consumption indicates the need for improvements in public water treatment and better sanitary conditions because these can be a source of not only *H. pylori* infections but also other water-borne pathogen infections.

Keywords: Epidemiology. *Helicobacter pylori*. Risk factors. Brazil.

INTRODUCTION

Helicobacter pylori is the primary etiologic agent involved in gastric diseases in humans with worldwide distributions[1]. In 2005, *H. pylori* was identified as a microbiologic contaminant of water, and its role in gastric diseases was further assessed[2-4]. Improvements in sanitary conditions and higher human development indices have reduced the prevalence rate of *H. pylori* infection in developed countries. However, the prevalence rate in developing countries remains high[1].

Populations with lower socioeconomic levels present with higher prevalence rates of *H. pylori* infection[5-7]. In developing countries, the prevalence rate is higher than 70%, whereas in developed countries, such as Canada, Australia, and Switzerland, the prevalence rate can be as low as 30%. The prevalence rate of *H. pylori* infection in Brazil, particularly in its poorest areas, is similar to that in Africa, which is around 70-90%[1,8].

Brazil has a population of over 200 million individuals with varying ethnic and geographic backgrounds. The country is heterogeneous with respect to sanitary coverage, and a large proportion of its population still do not have access to high-quality water[9,10]. Access to high-quality water is not evenly distributed across Brazil and is highly dependent on sanitary facilities and infrastructure.

H. pylori is considered a carcinogen because it can induce the development of lesions such as atrophy, metaplasia, and dysplasia, leading to adenocarcinoma[11,12]. A meta-analysis showed that the risk of developing gastric cancer increased by two-fold when individuals were infected with *H. pylori* strains that contained the *cagA*+ gene[13-15]. The use of antibiotic therapy for

Corresponding Author: Drª Selma Maria Bezerra Jerônimo.
e-mail: selma.b.jeronimo@gmail.com
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the treatment of *H. pylori* infection decreased the development of gastric cancer, particularly when the treatment was started prior to the onset of precursor lesions. Controlling environmental factors, such as high salt intake, nitrites, and smoking, has decreased the burden of *H. pylori* infection[14]. Therefore, decreasing the risk factors of *H. pylori* infection is one of the measures that can prevent the development of gastric cancer. This study aimed to evaluate the risk factors associated with *H. pylori* infection in patients undergoing endoscopy to validate the cause of dyspeptic symptoms in an urban population in northeast Brazil and to compare the urease test and polymerase chain reaction (PCR) assay results with histopathological findings.

METHODS

Study participants

The study was conducted in Campina Grande, State of Paraiba, Northeast Brazil, a city with a population of 402,912 inhabitants. Both male and female participants and those aged 18 years and older who agreed to participate in the study were recruited from a referral center for gastric diseases. Participants were excluded from the study if they used nonsteroidal antiinflammatory proton pump inhibitors or antibiotics a month prior to examination or if they lived outside the City of Campina Grande. We did not include participants living outside Campina Grande because we could not validate the source of drinking water. Moreover, participants who were pregnant, those who underwent gastrectomy, or those who were unable to undergo endoscopy were excluded. Participants were asked to complete a survey questionnaire with demographic, social, economic, and lifestyle questions. Of the 1,257 participants scheduled to undergo endoscopy between February 2012 and October 2012, 759 underwent endoscopy, of which 200 met the inclusion criteria and were recruited.

Endoscopy assessment

After clinical evaluation, the participants underwent endoscopy, which was performed by a trained endoscopist. Macroscopic data included topography, localization, and type of injury. Systemic anesthesia was conducted with midazolam and fentanyl. During the procedure, gastric biopsies were carried out (two in the antrum, two in the body, and one in the incisura angularis) for histological study, which is in accordance with the recommendations of the Brazilian Society of Digestive Endoscopy.

Determination of *Helicobacter pylori* infection

H. pylori infection was determined via histological examination, urease test, and PCR. Histological examination is the gold standard test for *H. pylori* infection. Two fragments were collected from the antrum to determine the presence of urease (Urotest Kit, RenyLab, Barbacena, MG, Brazil), and all test results were read within 24 hours of tissue collection.

Tissue fragments were fixed in 10% formalin and were prepared according to standard histology protocol. Slides were stained with hematoxylin-eosin and were analyzed by a single pathologist. Deoxyribonucleic acid (DNA) was extracted from formalin-fixed paraffin-embedded tissues obtained from gastric biopsy[16]. Eight slices (10μm) from each sample were collected, and DNA extraction was performed using QIAamp DNA from paraffin-embedded tissues (QIAGEN Inc., Chatsworth, California, the USA) according to the manufacturer's instructions. The presence of *H. pylori* DNA was confirmed using PCR amplification of the 16S-ribosomal DNA (rDNA) using specific primers (5′-CTGGAGAGACTAAGCCCTCC-3′ and 5′-GAGGAATACTCATTGCGAAGGCGA-3′][16]. PCR reactions were performed with 20μL containing 1× PCR buffer, 0.2 mmol/L of each deoxynucleotide, 0.2μM of each primer, and 1 unit of Taq polymerase. The PCR cycles were 95°C for 2 min; (95°C for 1 min; 58°C for 1 min; and 72°C for 1 min) 40X; 72°C for 10 min; and 10°C. Gel electrophoresis (2% agarose) was used to determine whether PCR products had the correct length of 229bp. Ten samples positive on PCR were sent for sequencing at Macrogen Inc. (Seoul, Korea) to confirm the results of the PCR assay.

Histopathological examination

The diagnoses were made according to the updated Sydney System for chronic gastritis when the presence of lymphocytes in the *lamina propria* was higher than expected[17]. The existence of plasma cells was considered an indicator of chronic inflammation. The presence of neutrophils was considered a marker of active gastritis. The loss of glandular tissue was considered a marker of gastric mucosal atrophy[17].

Ethical considerations

The study protocol was reviewed and approved by the ethical review board of Universidade Federal do Rio Grande do Norte. The study was conducted in accordance with the ethical standards of the Brazilian national committee of Ethics in inhuman research, which follows the principles of the Declaration of Helsinki. The approval number is CAAE: 0251.0.051.127-10. Informed consent was obtained from all the participants.

Statistical analysis

The comparisons were based on the *H. pylori* infection status, which was determined via histological examination. Fisher's exact and Wilcoxon rank-sum tests were used to compare categorical and continuous variables, respectively. The comparison of sensitivity and specificity between diagnostic methods (Urease vs PCR) were determined by McNemar's chi-square. Logistic regression models were established to further examine demographic, lifestyle, and socioeconomic factors associated with the risk of developing *H. pylori* infections. All analyses were performed using Stata (S*tata* Statistical Software: Release 11. College Station, TX, United States) with 95% confidence interval (CI), and we set the significance level (α) at 0.05.

RESULTS

Comparison of diagnostic methods for *H. pylori* infection

The prevalence rate of *H. pylori* infection was 69.5% (139/200), 60% (120/200), and 58% (116/200) via the urease test, histological examination, and PCR assay, respectively.

The urease test was more sensitive than PCR assay [92% (95% CI: 0.85, 0.96) vs. 82% (95% CI: 0.74, 0.89), p=0.012] for diagnosing *H. pylori* infection, whereas PCR assay had a higher specificity than the urease test [78% (95% CI: 0.67, 0.86) vs. 64% (95% CI: 0.52, 0.74), p=0.034]. **Table 1** shows the performances of the two methods with respect to histological examination, which is the gold standard test for *H. pylori* infection.

Demographic and clinical characteristics of the study population

All participants were from the same geographic region; 72.5% (145/200) of the participants were women. The sociodemographic data of the population are shown in **Table 2.** No difference was observed in the educational and socioeconomic levels of the *H. pylori*-positive or *H. pylori*-negative groups. Current smoking habits [adjusted odds ratio (OR): 5.19; 95% CI: 1.29-20.86; p=0.02] and intake of non-treated water (OR: 2.20; 95% CI: 1.02–4.72; p=0.044) were associated with *H. pylori* infection (**Table 3**). Other variables, such as age, hand washing, and cup sharing, were not associated with *H. pylori* infection (**Table 3**).

Helicobacter pylori infection and its relationship with gastric diseases

Gastritis and duodenal ulcer were common findings in both individuals with and without *H. pylori* infection. Belching was the only symptom (p=0.05) associated with *H. pylori* infection (**Table 4**). There was no association between *H. pylori* infection and a family history of either peptic ulcer (p=0.457) or gastric cancer (p=0.447) (**Table 4**). Mild gastritis was more frequently observed in individuals who were *H. pylori* negative (p<0.001) than in those with moderate or severe gastritis, which was commonly associated with *H. pylori*-positive individuals (p<0.001) (**Table 5**). No difference was observed between the two groups in terms of gastroduodenal ulcer (p=0.474). There was no association between alcohol intake (p>0.05) and the grade of inflammation. Cigarette smoking (p=0.020) and consumption of municipal water (p=0.044) were associated with mild and moderate gastritis.

DISCUSSION

This is the first study conducted in the State of Paraíba, Northeast Brazil that assessed the effect of sociodemographic factors on the development of *H. pylori* infection. This pathogen affects half of the world's population, and it exceeds 80% in developing countries[1]. The prevalence rate of *H. pylori* infection was higher in the population who used non-treated water. These results are consistent with those of studies conducted in other countries in South America and in the other regions of Brazil[1]. Although Brazil has the eighth largest economy, it still has epidemiological characteristics similar to those of developing countries, where the prevalence rate of *H. pylori* infection is higher than 80%. Zaterka et al. assessed 1,008 blood donors in São Paulo, and their results showed that the prevalence of *H. pylori* infection was also high, regardless of sex[18].

H. pylori infection was most commonly observed in adults with an average age of 40 years. The prevalence of the infection among elderly individuals in Brazil is usually high, which is greater than 68% of the cases. Our results are in accordance with those of other studies. Gasbarrini et al. assessed health professionals in Rome, Italy and found that individuals who are older and those with fathers with fewer years of education were more likely to be *H. pylori* positive[19]. Another study conducted in Kuwait revealed a higher prevalence in women younger than 30 years[20].

Income level, educational level, and household crowding were similar in our study population. The association between an increased risk of *H. pylori* infection and coffee consumption was not statistically significant. *H. pylori* infection was more common among smokers and drinkers, regardless of

TABLE 1: Performance of the urease test and PCR for the diagnosis of *Helicobacter pylori* using histological examination as the gold standard test.

Test	Histological examination		Total
	negative	positive	
Urease			
negative	51	10	61
positive	29	110	139
total	80	120	200
PCR			
negative	62	21	83
positive	18	98	116
total	80	119*	199

PCR: polymerase chain reaction. Urease: sensitivity = 110/120 = 92% and specificity = 51/80 = 64%. PCR: sensitivity = 98/119* = 82% and specificity = 62/80 = 78%. *One sample failed in the PCR assay.

TABLE 2: Characteristics of the study population in accordance to *Helicobacter pylori* infection.

Characteristics of the participants	*Helicobacter pylori* negative (n=80)	*Helicobacter pylori* positive (n=120)	p value*
Sex, n (%)			
male	19 (23.8)	36 (30.0)	0.419
female	61 (76.2)	84 (70.0)	
Age group (years), n (%)			
18–39	22 (27.5)	59 (49.2)	**0.004**
40–59	38 (47.5)	46 (38.3)	
≥60	20 (25.0)	15 (12.5)	
Median age (IQR), years	47 (38–59.5)	40 (32.5–52.0)	**0.005**
Median BMI (IQR), kg/m^2	26.0 (23.1–30.1)	26.4 (23.5–30.2)	0.741
Education, n (%)			
low	40 (50.0)	44 (36.7)	
medium	23 (28.8)	43 (35.8)	0.179
high	17 (21.2)	33 (27.5)	
Income, n (%)			
low	9 (11.2)	13 (10.8)	
medium	61 (76.3)	96 (80.0)	0.738
high	10 (12.5)	11 (9.2)	
Household crowding, n (%)			
<1 person/room	60 (75.0)	78 (65.0)	0.09
≥1 person/room	20 (25.0)	42 (35.0)	
Smoking status, n (%)			
never	50 (62.5)	74 (61.7)	
current	3 (3.8)	18 (15.0)	**0.018**
former	27 (33.7)	28 (23.3)	
Alcohol drinking, n (%)			
never	43 (53.8)	62 (51.7)	
current	15 (18.7)	42 (35.0)	**0.009**
former	22 (27.5)	16 (13.3)	
Coffee drinking, n (%)			
yes	70 (87.5)	109 (90.8)	0.299
no	10 (12.5)	11 (9.2)	
Water supply, n (%)			
mineral water	31 (38.8)	22 (18.3)	
municipal	41 (51.2)	78 (65.0)	**0.005**
others	8 (10.0)	20 (16.7)	
Hand washing, n (%)			
yes	66 (82.5)	84 (70.0)	**0.032**
no	14 (17.5)	36 (30.0)	
Cup sharing, n (%)			
yes	51 (63.8)	84 (70.0)	0.220
no	29 (36.2)	36 (30.0)	

IQR: interquartile range; **BMI**: body mass index. *Fisher's exact test was used for categorical variables, and Wilcoxon rank-sum test was utilized for. quantitative variables.

TABLE 3: Risk factors of *Helicobacter pylori* infection.

	Unadjusted			Adjusted*		
Variable	**OR**	**95% CI**	**p value**	**OR**	**95% CI**	**p value**
Sex						
male	1.00	-	-	1.00	-	-
female	0.73	(0.38–1.39)	0.333	0.68	(0.31–1.56)	0.350
Age	0.98	(0.96–0.99)	**0.011**	0.98	(0.96–1.01)	0.232
BMI	1.00	(0.95–1.06)	0.918	1.03	(0.97–1.11)	0.317
Education						
low	1.00	-	-	1.00	-	-
medium	0.96	(0.44–2.09)	0.924	0.84	(0.34–2.06)	0.703
high	0.57	(0.27–1.17)	0.125	0.66	(0.25–1.73)	0.398
Income						
low	1.00	-	-	1.00	-	-
medium	1.43	(0.57–3.57)	0.443	0.95	(0.30–2.99)	0.934
high	1.31	(0.39–4.39)	0.658	0.66	(0.15–2.98)	0.590
Household crowding						
<1 person/room	1.00	-	-	1.00	-	-
≥1 person/room	1.62	(0.86–3.03)	0.136	1.22	(0.60–2.48)	0.579
Smoking status						
never	1.00	-	-	1.00	-	-
current	4.05	(1.13–14.5)	**0.031**	5.19	(1.29–20.86)	**0.020**
former	0.70	(0.37–1.33)	0.275	1.05	(0.49–2.24)	0.904
Alcohool intake						
never	1.00	-	-	1.00	-	-
current	1.94	(0.96–3.93)	0.066	1.47	(0.65–3.33)	0.361
former	0.50	(0.24–1.07)	0.075	0.45	(0.18–1.10)	0.079
Coffee drinking						
no	1.00	-	-	1.00	-	-
yes	1.42	(0.57–3.51)	0.453	1.30	(0.45–3.81)	0.630
Water supply						
mineral water	1.00	-	-	1.00	-	-
municipal	2.68	(1.38–5.21)	**0.004**	2.20	(1.02–4.72)	**0.044**
others	3.52	(1.32–9.44)	**0.012**	3.87	(1.30–11.47)	**0.015**
Hand washing						
yes	1.00	-	-	1.00	-	-
no	2.02	(1.01–4.05)	**0.048**	1.80	(0.81–3.99)	0.150
Cup sharing						
no	1.00					
yes	1.33	(0.73–2.42)	0.356	1.17	(0.57–2.40)	0.664

OR: odds ratio**; 95% CI:** confidence interval 95%.*The multiple logistic regression model included all variables mentioned in **Table 3** as covariates.

whether they were light or heavy consumers. Smoking has been considered a risk factor of *H. pylori* infection[18,21,22]. The association between *H. pylori* infection and smoking has been attributed to socioeconomic conditions. Furthermore, smoking is a major risk factor for peptic ulcer disease because it stimulates gastric acid secretion and compromises the mucosal barrier, resulting in gastric atrophy and potentially leading to the development of cancer[23]. The association between two or more risk factors and *H. pylori* infection in the same patient also increases the risk of ulcer development[24]. Chen et al. showed that smoking was associated with an increased risk of duodenal ulcer in patients with *H. pylori* infection[25]. Infection stimulates the production of pepsinogen and decreases mucous production, leading to more pathology. *H. pylori* infection was common among drinkers. However, no association was found after controlling for confounders, as observed in a previous study[26].

TABLE 4: Clinical findings in accordance to *Helicobacter pylori* infection.

Clinical findings	*Helicobacter pylori* negative (n=80)	*Helicobacter pylori* positive (n=120)	p value*
Symptoms, n (%)			
nausea	20 (25.0)	34 (28.3)	0.362
vomiting	11 (13.8)	13 (10.8)	0.341
heartburn	49 (61.3)	83 (69.2)	0.157
epigastric pain	57 (71.3)	92 (76.7)	0.243
loss of appetite	12 (15.0)	16 (13.4)	0.447
early satiety	6 (7.5)	9 (7.5)	0.602
belching	11 (13.8)	29 (24.2)	**0.050**
regurgitation	5 (6.3)	12 (10.0)	0.254
hiccups	7 (8.8)	11 (11.2)	0.565
fullness	36 (45.0)	52 (43.4)	0.465
globus	10 (12.5)	15 (12.5)	0.582
Family history, n (%)			
peptic ulcer	20 (25.0)	28 (23.3)	0.457
gastric cancer	12 (15.0)	16 (13.3)	0.447

*Each symptom was compared in a 2×2 table using one-sided Fisher's exact test.

TABLE 5: Microscopy (histology) findings considering the presence of *Helicobacter pylori.*

Microscopic findings, n (%)	*Helicobacter pylori** negative (n=80)	*Helicobacter pylori** positive (n=120)	p value**
intestinal metaplasia	12 (15.0)	10 (8.3)	0.107
gastric atrophy	9 (11.3)	24 (20.0)	0.073
mild gastritis	38 (47.5)	18 (15.0)	<0.001
moderate gastritis	24 (30.0)	67 (55.8)	<0.001
severe gastritis	2 (2.5)	33 (27.5)	<0.001

*Participants who had multiple symptoms. **Each finding was compared in a 2×2 table using Fisher's exact test.

In our study, individuals who consumed non-treated water and did not wash their hands before meals were more likely to acquire the infection. Sharing of cups among family members was not associated with the development of the infection. *H. pylori* is considered to be one of the microbiological contaminants of water. The viability of the bacteria is dependent on temperature conditions, which are ideal in the tropics, and this strengthens the assumption that contaminated water can be a natural source of infection[4,27]. The presence of *H. pylori* in non-treated water samples has been reported in other studies[10]. Campina Grande ranked 29th in water quality in Brazil, and it still does not have complete sewage coverage. However, because of frequent water shortage, individuals are more likely to store water at home, and the risk of contamination becomes higher. Hand washing has contributed to a reduction in infections, particularly in the gastrointestinal tract[18,28].

Belching was associated with *H. pylori* infection, although it has a borderline p value. Dyspepsia, which is defined as pain or discomfort in the upper abdomen, is the main clinical manifestation in most patients. Usually, this symptom causes participants to seek medical help, and these individuals are then required to undergo endoscopy[11]. Moreover, heartburn and epigastric pain were symptoms commonly associated with the persistence of *H. pylori* infection in human immunodeficiency virus-positive individuals[29]. Inflammation of the gastric mucosa increased with exposure to the bacteria. We found more *H. pylori*-positive patients with chronic gastritis, and this result is in accordance with the findings showing that chronic infection increases the severity of gastritis[30].

In conclusion, the prevalence rate of *H. pylori* was high in patients dyspeptic disorders and was associated with the severity of gastritis, and this increased the awareness of the need to improve the city's non-treated water as a primary intervention to decrease *H. pylori* infection. PCR is a sensitive and specific diagnostic method that can be implemented in pathology

laboratories and can be used as a confirmatory assay for *H. pylori* infection. In addition, it can help identify the genotype strains of *H. pylori* that contain the *cagA+* gene associated with neoplastic development[13].

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Conflict of interest

The authors declare that there is no conflict of interest.

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