

## Relationship, association and distribution of *Helicobacter pylori* infection in endoscopy patients at a Tertiary Hospital in Makurdi, Benue State, Nigeria to the symptoms of nausea and vomiting

Mnena Eunice Yaji\*, Terdzungwe Thaddaeus Sar and Paulyn Tracy Aernan

Department of Microbiology, Joseph Sarwuan Tarka University Makurdi, Benue State, Nigeria.

World Journal of Advanced Research and Reviews, 2024, 21(02), 790–795

Publication history: Received on 23 December 2023; revised on 29 January 2024; accepted on 01 February 2024

Article DOI: <https://doi.org/10.30574/wjarr.2024.21.2.0391>

### Abstract

The distribution of *H. pylori* obtained from patients referred for endoscopy at Benue State University Teaching Hospital (BSUTH) Makurdi, Benue State, Nigeria in relation to nausea and vomiting was examined in this study. To isolate *H. pylori*, gastric biopsy samples were obtained from the antrum of 80 consenting patients, and questionnaires were administered to them. The biopsies were placed in Brain Heart Infusion (BHI) broth and were used for isolation. Genomic Deoxyribonucleic Acid (gDNA) was extracted from the biopsies using ReliaPrep spin column method and used for singleplex Polymerase Chain Reaction (PCR). Out of the 80 patients, 24 (30%) had *H. pylori* infection as detected by 16S rRNA PCR. There was no significant association between infection and nausea/vomiting (OR = 5.622, 95% CI: 0.683-46.274;  $p = 0.076$ ), or infection and hematemesis, common symptoms reported by patients (OR = 0.911, 95% CI: 0.304 - 2.729;  $p = 0.870$ ). The research sheds light on the infection, prevalence and distribution of *H. pylori* in endoscopy referral patients at BSUTH, with respect to nausea and vomiting, and provides valuable information for both clinicians and researchers.

**Keywords:** *Helicobacter pylori*; Endoscopy; Distribution; Nausea; Vomiting; Benue State; Nigeria

### 1. Introduction

*Helicobacter pylori* (*H. pylori*) is a Gram-negative microaerophilic bacterium which colonizes the gastric mucosa of more than half of the world's population [1]. *H. pylori* infection may be acquired during childhood and persists as a life-long infection in the absence of treatment. It causes diseases such as chronic gastritis; peptic ulcer; gastric malignancies and gastric mucosa-associated lymphoid tissue (MALT) lymphoma [2;3]. *H. pylori* is recognized as a Class I carcinogen by the International Agency for Research on Cancer [4] and is considered one of the strongest known risk factors for gastric malignancies [5;6;7;8]. Approximately 89% of all gastric cancers are attributed to *H. pylori* infection; and the eradication of this infection has been known to reduce gastric cancers [9;10]. Gastric cancer is ranked sixth in incidence and second in mortality among all cancers worldwide based on recent global cancer statistics in 2018 [11]. The probable routes of transmission of *H. pylori* are faecal-oral and intra-familial [12]; thus rendering the risk factors for *H. pylori* infection closely associated with food and personal hygiene. Other known risk factors associated to *H. pylori* infection include age; socioeconomic status; number of siblings; household crowding; ethnicity; migration from high prevalence regions; infection status of family members; and sanitary facilities [13;14;15].

Though the epidemiology of *H. pylori* infection has changed with improvements in sanitation and methods of eradication; the prevalence of *H. pylori* is still high in developing countries; and ranges between 85% and 95%; while it is between 30 and 50% in developed countries [16]. The high prevalence in the former is related to socioeconomic status and levels of hygiene.

\* Corresponding author: Yaji Mnena E

Despite the global recognition of *H. pylori* as a major public health concern; there is paucity of information on its prevalence and distribution in specific Nigerian populations. Nigeria has unique socio-economic and environmental factors, and the prevalence and distribution of *H. pylori* are crucial and warrant investigation; especially among patients referred for endoscopy. This study seeks to address the information and data gap and link the distribution of *H. pylori* infection to nausea and vomiting by focusing on patients at Benue State University Teaching Hospital (BSUTH) Makurdi; Benue State; Nigeria

---

## 2. Material and methods

### 2.1. Study Area

Benue State is located in the north-central geopolitical region in Nigeria and shares boundary with five other states; namely, Nasarawa to the North, Taraba to the East, Cross-River to the South, Enugu and Ebonyi to the South-West and Kogi to the West. The state also shares a common boundary with the Republic of Cameroun to the South-East. Makurdi, situated on the Bank of the Benue River, Makurdi is the Benue State Capital. The cosmopolitan town lies between latitude 07° 45'N and longitude 08° 32' E; with a mean elevation of 92 meters above sea level [19].

### 2.2. Ethical Approval and Patient Recruitment

Ethical approval was obtained from the Health Research Ethics Committee of the BSUTH; Makurdi; where the study was carried out. All participants had medical referrals for gastric biopsy at the Gastroenterology Department of the BSUTH, Makurdi. Volunteer participants were informed of the details of the study and consented before being enrolled into the study.

Patients were recruited from the Gastroenterology Unit of the BSUTH, Makurdi. Subjects were patients that had various *H. pylori*-associated dyspeptic symptoms including epigastric pain, fullness, vomiting, nausea and flatulence. A Gastroenterologist performed the endoscopy on informed and consenting participants.

### 2.3. Sample Size Determination

Sample size was calculated using Raosoft (2018) Sample Size Calculator. At 0.05 alpha level of significance; 95% confidence level and a patient population size of 99 and previous prevalence 50%; a sample size of 80 was obtained.

#### 2.3.1. Inclusion Criteria

- Patients with dyspepsia
- Patients who required endoscopy as part of diagnosis
- Patients from who informed consent was obtained

#### 2.3.2. Exclusion Criteria

- Patients without symptoms of dyspepsia

### 2.4. Questionnaires

Questionnaires on the characteristics of the epigastric pain and symptoms were administered to volunteer participants

### 2.5. Genomic DNA (gDNA) Extraction

The Genomic DNA was extracted using ReliaPrep gDNA miniprep kit (Promega; Southampton; UK).

The 2 ml tubes to be used were selected and labeled, and 200 $\mu$ l of specimen were placed in them. Proteinase K (25 $\mu$ l) was dispensed into the tubes and the contents mixed by vortexing for one minute to destroy other proteins and release the bacterial DNA. Lysis buffer (200 $\mu$ l) was added and mixed by vortexing for 10 seconds and incubated at 56°C for 10 minutes in a water bath to help the buffer work maximally. Binding buffer (250  $\mu$ l) was added to each tube and mixed for 10 seconds. Spin columns were selected, labelled and placed in collection tubes. The lysates were transferred into the corresponding spin columns with silica membrane and binding buffer to help release DNA and to adsorb to the silica membrane. The columns were centrifuged at 14000 revolutions per minute (rpm), the flow through discarded, and new collection tubes inserted. Column wash buffer (500 $\mu$ l) was used to wash the columns three times and centrifuged for 3 minutes at 14000 rpm. The flow through was discarded at each step. Spin columns were placed in clean collection tubes and centrifuged (14000 rpm; 1 min) to remove residual wash buffer and placed in clean 1.5ml recovery tubes. Sterile

nuclease free water (200  $\mu$ l) was added into each tube and incubated at room temperature for one minute and then centrifuged (13000 rpm; 1 min). The DNA quality was checked at 260/280nm using Eppendorf Biophotometer Plus (Eppendorf; Germany). Nuclease free water was used as blank. Values of 1.2 and above were regarded as pure. The genomic DNA was labelled and used for further tests immediately and the remaining stored in the fridge for further use.

## 2.6. Specific Primers for Detection of *H. pylori*

The forward primers 16S rRNA – F and the reverse primers 16S rRNA – R were spun to bring the powder to the bottom of the vial.

### 2.6.1. The primer sequences were

16S rRNA –F = GGAGGATGAAGGTTTTAGGATTG (23); and

16 rRNA - R = TCGTTTAGGGCGTGGACT [18].

(Urofins mwg/operon Germany) as described by Chamanrokh *et al.* [20]. Water was used as No template control; *E. coli* DNA sample as negative control and *H. pylori* samples as positive control. The 1 x TE solution at pH8 was used to dilute the primer. Tris EDTA (Ethylenediamine Tetraacetic acid) (643  $\mu$ l) was used to dilute the forward primer 16S rRNA – F and Tris EDTA (924  $\mu$ l) added to the reverse primer 16S rRNA –R and was allowed to dissolve in it for a few minutes. The mixture was vortexed for a few seconds and centrifuged for 1 minute. Stock (100  $\mu$ l) was made and 50  $\mu$ l of the stock (both forward and reverse Primers) were put into 450  $\mu$ l of the 1 x TE buffer solution and mixed thoroughly. RNase free water (550  $\mu$ l) was added to 100  $\mu$ l of both forward and reverse primers to give the primer mix. PCR plates in a PCR rack containing PCR master mix (12.5  $\mu$ l); primer mix (7.5  $\mu$ l) and gDNA (5.0  $\mu$ l) were each mixed, capped and placed in the PCR machine and run at the thermal profile of 95°C for two minutes; 94°C for 18 seconds; 60°C for 30 seconds; 72°C for 30 seconds and 72°C for 5 minutes, for 40 cycles making a total of 1h 30 min at which 294 bp product size was amplified; brought out of the machine and loaded into 2.0 % agarose gel wells in the electrophoresis tank containing 1 x TBE buffer. The DNA ladder with 100 bp was placed into the first well to act as a ruler. Loading dye (3  $\mu$ l) which helps to sink the DNA into the gel was mixed with the specimens and 20  $\mu$ l of the mixture was loaded into the remaining wells. The electrophoresis tank was covered; connected to a power source at 100 V for 30 minutes; and was read under ultraviolet (UV) light. Images were captured using GenoMini Electrophoresis Gel (VWR; UK) system.

## 2.7. Statistical Analysis

Data obtained from the study were analysed using Statistical Package for Social Sciences (SPSS) version 20 (IBM Inc.) Odds ratio was used to assess risk factors. Alpha level of significance was set at 0.05.

## 3. Results

Out of the eighty (80) biopsy samples collected; 24 (30%) had *H. pylori* as detected by PCR of the 16S rRNA gene (Table 1).

For nausea and vomiting; no significant association was found between infection and nausea/vomiting (OR = 5.622; 95% CI: 0.683-46.274; p = 0.076); infection and vomiting blood (hematemesis) (OR = 0.911; 95% CI: 0.304-2.729; p = 0.870) were noted (Table 2).

**Table 1** Distribution of *Helicobacter pylori* by PCR of the 16S rRNA Method (n=80)

Test	Frequency (%)
PCR (16S rRNA gene)	
Positive	24(30)
Negative	56(70)

**Table 2** Distribution of *Helicobacter pylori* Infection in Relation to Nausea and Vomiting

Variables	Number (%)	Number Positive (%)	Number Negative (%)	Chi-Square value	P-value
Signs and symptoms					
Nausea and Vomiting					
Yes	68(85)	23(33.82)	45(66.18)	3.160	0.076
No	12(15)	1(8.33)	11(91.67)		
Total	80(100)	24	56		
Vomiting blood					
Yes	21(26.25)	6(28.57)	15(71.43)	0.028	0.868
No	59(73.75)	18(30.51)	41(69.49)		
Total	80(100)	24	56		

#### 4. Discussion

The high prevalence of *H. pylori* infection as detected by PCR of the 16S rRNA gene is similar to the results based on seroprevalence that were reported among patients with gastritis in Kaduna and Enugu States in Nigeria [21; 22]. Similarly, previous studies based on histology of biopsies for *H. pylori* infection in Ibadan Western Nigeria [23] and [24] in Maiduguri, Northern Nigeria detected *H. pylori* from histology of gastric biopsies among gastritis patients in Nigeria. The prevalence of *Helicobacter pylori* infection observed in our study agrees with the global context of varying prevalence rates in different populations [18].

The lack of association between *H. pylori* infection and presence of nausea and vomiting is at variance with the findings of Grooten *et al.* [25] who reported association in Netherlands and Amr *et al.* [26] in Washington. This may be because of variation in geographical locations. One of the causes of bloody vomit during *H. pylori* infection is the development of gastric ulcers [26]. Though during the study, the staging of symptoms was not investigated; it is possible that the patients sampled in this study had not gotten to the stage of ulcer development, when vomiting and/or nausea is prevalent as found in other studies.

#### 5. Conclusion

In this study it was found that there was a high prevalence of *Helicobacter pylori* in gastritis patients in Makurdi; Benue State of Nigeria, as determined by PCR of the 16S rRNA gene. However, *H. pylori* infection was not found to be associated with nausea, vomiting or hematemesis. These symptoms alone cannot therefore be used definitive diagnosis for *H. pylori* infection in Benue State, Nigeria.

#### Recommendations

- There is need to establish national consensus guidelines on the management of *Helicobacter pylori* infection in patients with gastritis and peptic ulcer disease.
- Moreover, education and enlightenment campaigns highlighting relevance of good hygiene and sanitation in form of seminars, pamphlets, posters, radio and television messages or group discussions and town criers especially in villages need to be stepped up encouraged to help reduce infection rates.
- Future investigations should investigate the specific risk factors that contribute to *H. pylori* distribution in our population and explore potential interventions to mitigate its impact on patient health.

#### Compliance with ethical standards

##### Disclosure of conflict of interest

No conflict of interest to be disclosed.

*Statement of informed consent*

Informed consent was obtained from all individual participants included in the study

**References**

- [1] Hooi; J.K.Y.; Lai; W.Y.; Ng; W.K.; Suen; M.M.Y.; Underwood; F.E.; Tanyingoh; D.; Malfertheiner; P.; Graham; D.Y.; Wong; V.W.S. and Wu; J.C.Y. (2017). Global Prevalence of *Helicobacter pylori* Infection: Systematic Review and Meta-Analysis. *Gastroenterology*. 153:420–429
- [2] Cover; T.L. and Blaser; M.J. (2009). *Helicobacter pylori* in health and disease. *Gastroenterology*. 136:1863–1873
- [3] Muhammad; J.S.; Sugiyama; T. and Zaidi; S.F. (2013). Gastric pathophysiological ins and outs of *Helicobacter pylori*: A review. *Journal of Pakistan Medical Association*. 63:1528–1533
- [4] International Agency for Research on Cancer (IARC) (1994). Schistosomes; Liver Flukes and *Helicobacter pylori*. IARC; Lyon; pp. 1–241
- [5] Carrasco; G. and Corvalan; A.H. (2013). *Helicobacter pylori*-Induced Chronic Gastritis and Assessing Risks for Gastric Cancer. *Gastroenterology Research and Practice*. (10)1155-393015
- [6] Uemura; N.; Okamoto; S.; Yamamoto; S.; Matsumura; N.; Yamaguchi; S.; Yamakido; M.; Taniyama; K.; Sasaki; N.; Schlemper; R.J (2001). *Helicobacter pylori* infection and the development of gastric cancer. *New England Journal of Medicine*. 345:784–789
- [7] Graham; D.Y. (2015). *Helicobacter pylori* update: gastric cancer; reliable therapy; and possible benefits. *Gastroenterology*. 148:719–731
- [8] Muhammad; J.S.; Eladl; M.A. and Khoder; G. (2019). *Helicobacter pylori*-induced DNA Methylation as an Epigenetic Modulator of Gastric Cancer: Recent Outcomes and Future Direction. *Pathogens*. 2019;8:23
- [9] Yeh; J.M.; Kuntz; K.M.; Ezzati; M. and Goldie; S.J. (2009). Exploring the cost-effectiveness of *Helicobacter pylori* screening to prevent gastric cancer in China in anticipation of clinical trial results. *International Journal of Cancer*. 124:157–166
- [10] Takenaka; R.; Okada; H.; Kato; J.; Makidono; C.; Hori; S.; Kawahara; Y.; Miyoshi; M.; Yumoto; E.; Imagawa; A. and Toyokawa; T. (2007). *Helicobacter pylori* eradication reduced the incidence of gastric cancer; especially of the intestinal type. *Alimentary Pharmacology Therapeutics*. 25:805–812
- [11] Bray; F.; Ferlay; J.; Soerjomataram; I.; Siegel; R.L.; Torre; L.A. and Jemal; A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: Cancer Journal for Clinicians*. 68:394–424
- [12] Schwarz; S.; Morelli; G.; Kusecek; B.; Manica; A.; Balloux; F.; Owen; R.J.; Graham; D.Y.; van der Merwe; S.; Achtman; M. and Suerbaum; S. (2008). Horizontal versus familial transmission of *Helicobacter pylori*. *Public Library of Science Pathogens*. 4:e1000180
- [13] Forman; D.; De Backer; G.; Elder; J.; Moller; H.; Damotta; L.C.; Roy; P.; Abid; L.; Tjonneland; A.; Boeing; H. and Haubrich; H. (1993). Epidemiology of; and risk factors for *Helicobacter pylori* infection among 3194 asymptomatic subjects in 17 populations. *Gut*. 34:1672–1676
- [14] Graham; D.Y.; Malaty; H.M.; Evans; D.G.; Evans; D.J.; Jr.; Klein; P.D.; Adam; E (1991). Epidemiology of *Helicobacter pylori* in an asymptomatic population in the United States. Effect of age; race; and socioeconomic status. *Gastroenterology*. 100:1495–1501
- [15] Venneman; K.; Huybrechts; I.; Gunter; M.J.; Vandendaele; L.; Herrero; R. and Van Herck; K. (2018). The epidemiology of *Helicobacter pylori* infection in Europe and the impact of lifestyle on its natural evolution toward stomach cancer after infection: A systematic review. *Helicobacter*. 23:e12483
- [16] Muhammad; J.S.; Zaidi; S.F.; Saeed; S.A.; Ishaq; M (2017). Current status of *Helicobacter pylori* association with haematological and cardiovascular diseases: A mini review. *Journal of Pakistan Medical Association*. 67:907–911
- [17] Marshall; B. J.; & Warren; J. R. (1984). Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *The Lancet*; 323(8390); 1311-1315

- [18] Hooi; J. K. Y.; Lai; W. Y.; Ng; W. K.; Suen; M. M. Y.; Underwood; F. E.; Tanyingoh; D. and Malfertheiner; P. (2017). Global prevalence of *Helicobacter pylori* infection: systematic review and meta-analysis. *Gastroenterology*; 153(2); 420-429
- [19] Benue State AIDS control Agency (BENSACA) (2009). Benue State HIV/AIDS Epidemiology Response and policy synthesis 9pp
- [20] Chamanrokh; P.; Mohammad; H.S.; Mahnaz; M.S.; Taher; N. and Davood; E. (2015). Three Tests Used to Identify Non-culturable Forms of *Helicobacter pylori* in Water Samples. *Journal of Microbiology*; 8(4): 16811
- [21] Nwodo; E.N.; Yakubu; S.E.; Jatau; E.D. and Yaboya A (2009). Seroprevalence of *Helicobacter pylori* infection in patients with Gastritis and Peptic Ulcer Disease in Kaduna; Kaduna State; Nigeria. *African Journal of Basic and Applied Sciences* 1(56): 123-128
- [22] Neri; G.P.; Raymond; A.A.; Nora; C.U.; Uzoma; C. M. and Chinyere; P.N (2009). *Helicobacter pylori* prevalence in patients with upper G.I symptoms in Enugu Metropolis. *Nigerian Journal of Gastroenterology and Hepatology* 1: 37 – 49
- [23] Jemilohon; A.C.; Otegbayo; J.A.; Ola; S.O.; Oluwasola; O.A. and Akere; A. (2010). Prevalence of *Helicobacter pylori* among Nigerian patients with dyspepsia in Ibadan. *The Pan African Medical Journal* 6:18
- [24] Olokoba; A.B.; Gashau W.; Bwala S. and Adamu A. (2013). *Helicobacter pylori* Infection in Nigerians with Dyspepsia *Nigerian Medical Journal* 47(2): 79-81
- [25] Grooten; I. J.;Den Hollander; W. J.; Roseboom; T. J.; Kwipers; E.J.; Jaddoe; V. W.; Gaillard; R. and Raintier; R. C. (2017). *Helicobacter pylori* infection: a predictor of vomiting severity in pregnancy and adverse birth outcome. *Elvsevier*. 216(5):512-513
- [26] Amr; H. M.; Patrick; C. M.; Bradley; A. F. and Dustin; E.B. (2023). *Helicobacter pylori* exposure in nausea and vomiting of pregnancy increases risk of preterm delivery. *Infectious Diseases in Obstetrics and Gynecology*; (8):1-2