ORIGINAL ARTICLE ASIAN JOURNAL OF MEDICAL SCIENCES

A study on phenotypic characterization and genotypic determination of drug resistance of *Helicobacter pylori* from cases of acid peptic disease in a tertiary care center

Palaneswamy Savetha[1], Rajesh Samuel Ajit George[2], Ravin Devasir Sathyaseelan[3], Malini Evangeline Rose C[4], Noor Mohamed Rasik B[5]

[1,4]Assistant Professor, Department of Microbiology, Tagore Medical College and Hospital, Chennai, Tamil Nadu, [2]Assistant Professor, Department of Otorhinolaryngology. Sri Sathya Sai Medical College and Research Institute, Puducherry, [3]Professor, Department of General Medicine, [5]Assistant Professor, Department of Community Medicine, Sri Lalithambigai Medical College and Hospital, Faculty of Medicine, Dr. M.G.R. Educational Research Institute, Chennai, Tamil Nadu, India

Submission: 04-08-2023 Revision: 02-12-2023 Publication: 01-01-2024

ABSTRACT

Background: *Helicobacter pylori* infection is one of the major risk factors for gastroduodenal disease, and invasive or non-invasive tests are available to detect it. **Aims and Objectives:** The study aims to identify *H. pylori* phenotypically and genotypically to identify drug-resistant *H. pylori* in suspected cases of peptic acid disorders at a tertiary care center. **Materials and Methods:** This 6-month prospective and observational study was performed at Saveetha Medical College and Hospitals. Patients' gastric biopsy samples were obtained from 105 individuals with gastroduodenal disorders who had upper gastrointestinal endoscopies at the Hospital. A fiber optic endoscope and biopsy forceps were used for the endoscopy. Biopsy samples were collected from the antrum and placed in Eppendorf tubes for fast urease, culture, gram stain, polymerase chain reaction (PCR), and histological analysis. **Results:** A total of 78 (74%) males and 27 (26%) females were among the 105 patients. Endoscopic findings imply gastritis, gastric ulcer, and duodenal ulcer in 74 patients (70.48%). Rapid urease test (RUT), gram stain, and histology revealed positive results in 20 cases. RUT showed that 30.47% of patients tested positive. Gram stain detected 25.1% of *H. pylori* infections, and 12 cases were histopathologically positive. By PCR, 21.62% of gastritis patients were positive for *H. pylori* infection. RUT and PCR give comparable results for detecting *H. pylori* infection, with a strong association between *H. pylori* infection and peptic ulcer disease. **Conclusion:** A combination of RUT and PCR is an effective diagnostic approach for *H. pylori* infection, allowing doctors to select the most appropriate medications for specific patients.

Key words: *Helicobacter pylori*; Drug-resistant; Peptic acid; Rapid urease test; Polymerase chain reaction; Gram stain

Access this article online

Website: http://nepjol.info/index.php/AJMS

DOI: 10.3126/ajms.v15i1.57358

E-ISSN: 2091-0576

P-ISSN: 2467-9100

Copyright (c) 2024 Asian Journal of Medical Sciences

This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.

INTRODUCTION

The identification of *Helicobacter pylori* and its significant role in gastric diseases originated from simple observations of spiral organisms in the stomach lining of humans and animals. Nowadays, *H. pylori* is a concern due to its direct association with gastroduodenal disorders. The prevalence of *H. pylori* exceeds 90% in developing countries, colonizing the stomachs of over half of the global population.[1] The prevalence rate varies depending on geographic location, socioeconomic status, personal hygiene, and age. Lifelong infection with *H. pylori* is a major risk factor for developing clinically significant diseases, including peptic ulcers, chronic gastritis, gastric carcinoma, and

Address for Correspondence:
Dr. Malini Evangeline Rose C, Assistant Professor, Department of Microbiology, Tagore Medical College and Hospital, Chennai, Tamil Nadu, India. **Mobile:** +91-96000 40121. **E-mail:** malinir67@gmail.com

mucosal-associated lymphoid tissue (MALT) lymphoma.[2,3] *H. pylori* can survive in the gastric mucosa for many years.[4] This bacterium possesses classical virulence factors, such as outer membrane protein, urease, cytotoxin-associated gene A, and vacuolating cytotoxin A.[5]

Several diagnostic tests are available to detect *H. pylori* infection, categorized as invasive and non-invasive techniques. However, the selection of tests depends on the laboratory's resources and the clinical situation, whether for diagnostic or eradication purposes. Invasive tests require an upper gastrointestinal endoscopy and biopsy material for the examination, including rapid urease tests (RUTs), histopathological evaluation, culture, and polymerase chain reaction. The RUT detects the presence of *H. pylori* by measuring its abundant urease enzyme. Smear evaluation of specimens is another rapid diagnostic method. Culturing *H. pylori* is challenging, expensive, and requires specialized media due to its meticulous nature. Although culture is highly specific, it is less sensitive.[6]

Polymerase chain reaction (PCR) is now widely employed to identify *H. pylori* in dental plaque samples, saliva, and tissue specimens. This method offers high specificity, quick results, and the ability to identify different strains of bacteria for pathogenic and epidemiologic studies. In addition, *H. pylori* has been increasingly developing drug resistance, particularly due to the widespread use of antibiotics such as Macrolides, quinolones, and Metronidazole. Endoscopic diagnostic procedures have been used less frequently. Alternative techniques include antibody detection methods and carbon-labeled urea breath tests (UBTs).[7] Due to, their low cost, speed, and patient acceptability, serological tests are often employed for screening in epidemiological research. The most common and accurate non-invasive test for identifying *H. pylori* infection is the UBT. It can be used as a screening tool to evaluate eradication therapy's efficacy and detect infection in children.[8,9]

The study's goals were to determine the prevalence of *H. pylori* infection in dyspeptic patients, to assess the utility of the RUT, histopathology, and PCR for diagnosis, and to identify the prevalence of clarithromycin-resistant strains in the population by investigating genetic mutations through molecular analysis.

Aims and objectives

The aim of this study was to phenotypic identification of *H. pylori* and genotypic determination of drug-resistant *H. pylori* from suspected cases of acid peptic diseases in a tertiary care center.

MATERIALS AND METHODS

After receiving approval from the Human Institutional Ethical Committee, this 6-month prospective and observational study was carried out in the Department of Microbiology at Saveetha Medical College and Hospitals. Gastric biopsy samples were taken from 105 individuals with gastroduodenal disorders who had upper gastrointestinal endoscopy at the hospital.

Inclusion criteria

Specimens from inpatients and outpatients attending the surgery department and suggestive of upper gastrointestinal diseases, gastric ulcers, duodenal ulcers, antral gastritis, gastric carcinoma, and MALT lymphomas were included in the study.

Exclusion criteria

Patients with previous gastric surgery and active bleeding were excluded from the study.

Patients were allowed to provide informed consent before the endoscopy. They were told to fast overnight, and the endoscopy was done with a fiber optic endoscope. The endoscope and biopsy forceps were thoroughly rinsed with water before being immersed in 2% of glutaraldehyde for 20 min. The equipment was cleaned again with sterile normal saline before collecting the specimens. Patients' throats were sprayed with lignocaine spray and positioned comfortably on their left side to reduce gagging. Before insertion into the mouth, the end of the endoscope was lubricated with lignocaine jelly. Patients were instructed to swallow until the scope reached the esophagus. The stomach and duodenum's internal tissues were inspected for ulcers, erosion, inflammation, discoloration, and abnormal growth. Three biopsy samples were collected using biopsy forceps, preferably from the lesion site and the stomach antrum.

Four biopsy samples were taken from the antrum, 2 cm from the pylorus, and placed in sterile Eppendorf tubes. For rapid urease testing, one sample was inoculated into urea broth. Another specimen was collected in normal saline for gram staining, another in 70% ethanol for PCR, and the last in 10% formalin for histopathological examinations.

The biopsy sample was added to the broth, and the tubes were incubated at 37°C for rapid urease testing. Positive results were reported in the tissue sample that became pink within 20 min, indicating a rise in pH caused by *H. pylori* ammonia production. The slides were air-dried, heat-fixed for gram staining, and stained with methyl violet, Gram's iodine, acetone, safranin, and water. After that, the slides

were blotted dry and examined using an oil immersion objective. *H. pylori* was identified as Gram-negative curved bacilli. One specimen was fixed in 10% formalin for histopathological examinations, and paraffin sections were prepared and stained with Hematoxylin and Eosin (H&E) to examine *H. pylori*. *H. pylori* detection in gastric biopsies often involves various staining techniques to improve diagnostic accuracy. While routine H& E staining can reveal heavy bacterial loads, special stains such as Giemsa, Gimenez, Steiner, Warthin Starry, PAS-AB, Toluidine blue, and immune stains are valuable for enhancing the sensitivity and specificity of *H. pylori* detection. However, in our study, only H& E staining was done. The HELINI *H. pylori* real-time PCR Kit, a nucleic acid amplification real-time PCR kit, was used to detect and quantify *H. pylori* in human biological samples. Specificity, dynamic range testing, and PCR testing were performed.

Detection of *H. pylori* using PCR

PCR was done using a Purefast Bacterial DNA (Tissue) mini spin purification kit, and it detected *H. pylori* by identifying the urea gene. Clarithromycin resistance gene and point mutations were detected by agarose gel electrophoresis.

Statistical analysis

Data collected from patients were entered into an MS Excel sheet for analysis. Patients were categorized based on demographic details and clinical conditions. Quantitative data were represented using percentages, figures, and tables. SPSS 21.0 was used for conducting the statistical analysis and data visualization.

RESULTS

Of the 105 patients examined with upper gastrointestinal symptoms, 78 (74%) were male and 27 (26%) were female.

The plurality (40%) of the cases studied were between the ages of 51 and 60, with 25% older than 61. Endoscopic examination indicated gastritis symptoms in 70.48% of the patients, including antral, fundal, diffuse, or erosive gastritis. Gastric ulcers were discovered in 16.19% of patients, while duodenal ulcers were found in 1.9%. Based on stomach growth, two individuals were diagnosed with gastric cancer. The remaining 9.52% of patients had normal results or other problems, such as polyps (Table 1).

The age group 51–60 years had the highest proportion of PCR-positive *H. pylori* cases (48.28%), followed by the 31–40 year group (17.25%). Most instances (3.4%) were reported in people under 20 (Table 2 and Figure 1).

Twenty cases were positive by RUT, gram stain, and histopathology, and seven cases were positive by RUT and gram stain alone. Five cases were positive by RUT and histopathology alone. All cases of RUT were positive and showed either gram stain or histopathology positive or both positive (Table 3).

About 30.47% of the cases (n=105) were positive by RUT. Seventeen samples tested by RUT showed positive in patients with gastritis. Out of 17 patients with gastric ulcer, 10 turned rapid urease positive. Duodenal ulcer and carcinoma showed 2 and 1 RUT positive About 25.1% of *H. pylori* infection was detected by Gram stain. Nine (52.94%) out of 17 patients with gastric ulcers were positive

Table 1: Demographic data of the study

Characteristics	Total	Percentage
Gender		
Male	78	74
Female	27	26
Age group (Years)		
11–20	2	1.9
21–30	9	8.5
31–40	15	14.3
41–50	10	9.5
51–60	42	40
>61	27	25.7
Endoscopic diagnosis		
Gastritis	74	70.48
Gastric ulcer	17	16.19
Duodenal ulcer	2	1.9
Carcinomatous growth	2	1.9
Others	10	9.52

Table 2: Categorization based on age for *H. pylori*-positive cases

Age group in years	No. of cases	*H. pylori*+ve (%)
<20	2	1 (3.4)
21–30	9	3 (10.34)
31–40	15	5 (17.24)
41–50	10	2 (6.9)
51–60	42	14 (48.28)
>61	27	4 (13.8)
Total	105	29 (100)

H. pylori: Helicobacter pylori

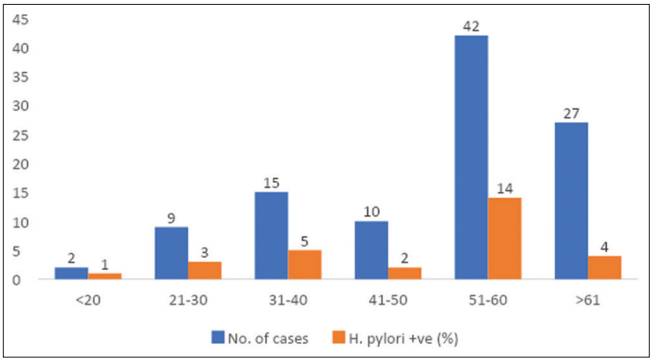

Figure 1: Age for *Helicobacter pylori*-positive cases

for Gram stain. Fifteen were positive in gastritis patients. Both patients with duodenal ulcers showed positive gram stains. One out of the two patients with carcinoma showed positive.

Histopathologically, 12 cases of *H. pylori* were detected in patients with gastritis and ten cases with stomach ulcers. Histopathology revealed positive results in both cases with duodenal ulcers. One out of every two cases of cancer tested positive. By PCR, 21.62% (16) of the 74 gastritis patients were positive for *H. pylori* infection. *H. pylori* was found in 52.94% and 50% of gastric and duodenal ulcer patients, respectively. *H. pylori* infection was found in one of the stomach cancer patients. *H. pylori* was found in two out of ten additional instances. There was a strong association between *H. pylori* infection and peptic ulcer disease compared to non-ulcer dyspepsia and CA stomach.

Rapid urease test and PCR gave comparable results for detecting *H. pylori*. Twenty-nine out of 32 RUT-positive samples were confirmed by PCR by detecting the specific *H. pylori* urea gene (Table 4 and Figure 1).

The amplification plots of a real-time quantitative PCR assay of a specific internal region of the urease A gene of *H. pylori* (Figure 2).

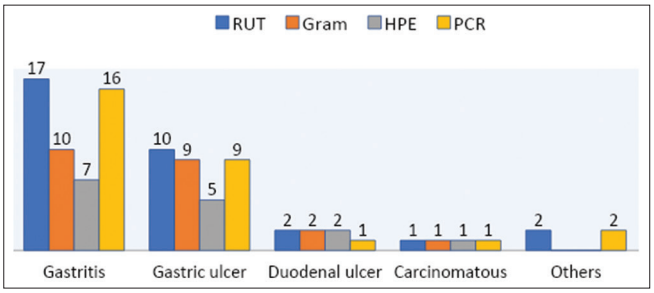

Figure 2: Urease and *Helicobacter Pylori*-positive in gram stain, histopathology, and polymerase chain reaction

Table 3: Correlation with combination of tests

Rapid urease test	Gram staining	Histopathology	No. of cases
+	+	+	20
+	+	-	7
+	-	+	5

We discovered one new point mutation at T2243C. We found no point mutations at locations 2142 or 2143. Five of the 29 isolates tested positive for mutation at the T2243C site of the 23S rRNA. Line 1 shows negative control, line 2 shows ladder 100 bp DNA, and S1 to S6 denotes the samples. Sample 5 shows the band at 288 bp fragment (S5) (Figures 3-5).

DISCUSSION

The study included 105 individuals with upper gastrointestinal symptoms, 74% of whom were men and 26% of whom were women. This observation is consistent with the findings of Subbukesavaraja and Balan[10] who discovered that 72.4% of biopsy specimens were from males and 27.6% from females. However, other research demonstrated that females have a higher prevalence of *H. pylori* infection. For instance, Khalifehgholi et al.,[11] reported female enrolment was higher than male enrolment. In our study, 72.41% of the 29 patients infected with *H. pylori* were male, whereas 27.89% were female, indicating a higher frequency of infection in male patients. This discovery agrees with the findings of Yoosuf et al., Archana et al., and Reddy et al.[12-14]

Most patients in this study were between the ages of 51 and 60. Although *H. pylori* infection is frequently associated with children, our study focused exclusively on adults. As a result, 75% of patients with gastrointestinal illnesses and *H. pylori* infection were between 30 and 70. A study by Reddy et al.,[14] had a similar distribution of cases. In our location, the prevalence of *H. pylori* in the gastric mucosa of dyspeptic patients was 27.61%, contrary to popular opinion in the Western world, where the prevalence is claimed to be 70–90% among Asians. Similar results were seen in studies by Chattopadhyay et al.,[15] (50%) from Kolkata and Archana et al.,[13] (48%) from Bagalkot. However, a higher prevalence of 58–70% was observed in a study by Arora et al.[16] The prevalence of infection can differ in various factors, including clinical condition, region and type of microorganisms, resistance patterns, and antibiotic susceptibility. Based on the literature, we have seen a variation in the age group of individuals

The endoscopic examination revealed that gastritis accounted for 70.4% of the cases, followed by gastric ulcer

Table 4: Comparison of urease with *Helicobacter pylori* positivity in gram stain, histopathology, and PCR

Clinical status	No. of cases	Rapid urease test	Gram stain	Histopathology	PCR
Gastritis	74	17	15	12	16
Gastric ulcer	17	10	9	10	9
Duodenal ulcer	2	2	2	2	1
Carcinomatous growth	2	1	1	1	1
Others	10	2	0	0	2
Total	105	32	27	25	29

PCR: Polymerase chain reaction

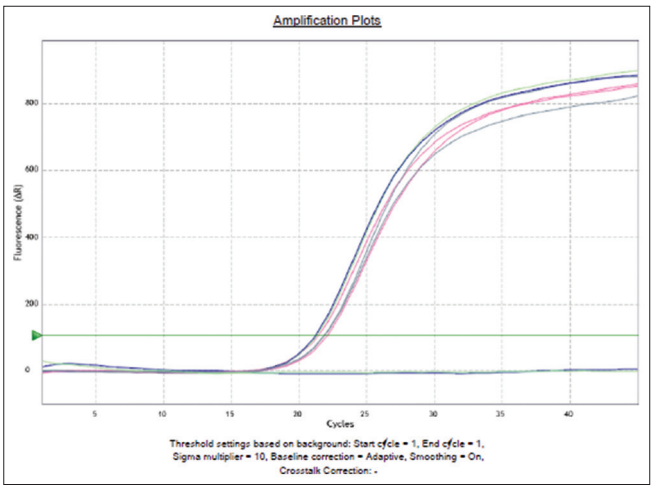

Figure 3: Amplification plots of internal controls

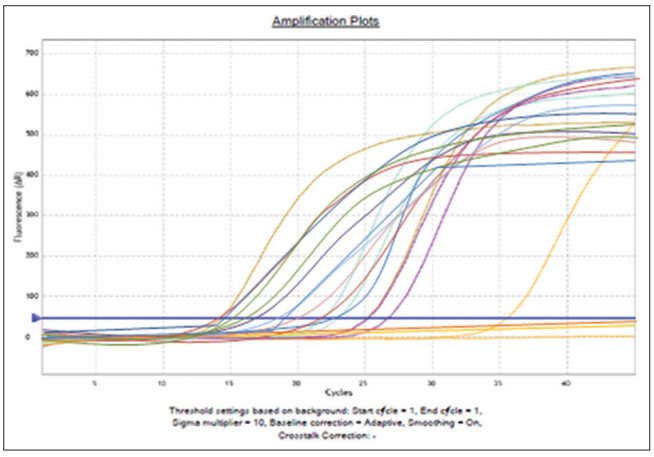

Figure 4: Amplification plots of *Helicobacter pylori* urea gene

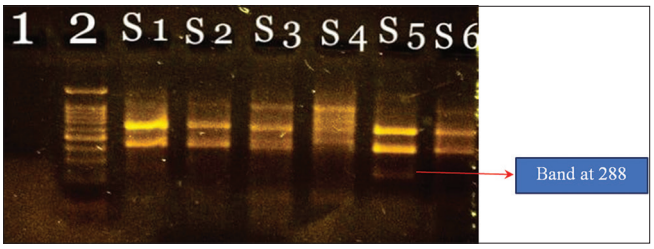

Figure 5: Agarose gel electrophotogram showing amplification performed *Helicobacter pylori* clarithromycin resistance gene from gastric biopsy samples

(16.19%), duodenal ulcer (1.9%), gastric carcinoma (1.9%), and other conditions (9.52%). A study by Subbukesavaraja and Balan[10] showed that duodenal ulcers accounted for 36%, gastritis for 30%, gastric carcinoma for 16%, and gastric ulcers for 17%. In our study, patients with gastric ulcers had the highest prevalence of *H. pylori* infection (52.94%), followed by duodenal ulcers and stomach cancer (50%). The prevalence of *H. pylori* gastritis was 21.62%. *H. pylori* infection was associated with peptic ulcer disease rather than non-ulcer dyspepsia, which is consistent with prior research conducted in India by Reddy et al.[14] Only two cases of gastric cancer were found in our analysis; one of them (50%) had *H. pylori* activity. Similarly, *H. pylori* activity was seen in three out of five cancer cases in research by Shrestha et al.[17]

Of the 105 samples tested using the RUT, 30.4% were positive. Similar results were seen in the findings Sivaprakash R et al., reported[12] (38.7%). Gram-stained smears (direct) in our study demonstrated a positivity rate of 25.7%, similar to the study conducted by Arora et al.,[16] and Subbukesavaraja and Balan,[10] who reported 20% positivity. However, Archana D et al.,[13] reported a higher positivity rate of 72.3%. PCR found *H. pylori* in all five histopathologically negative biopsy samples and four biopsy specimens that were Gram stain-negative but RUT positive in both cases. PCR found *H. pylori* in 20 samples, with positive results for RUT, Gram stain, and histopathology. According to Clayton et al.,[18] conventional tests reported negative reports in seven of the 23 PCR-positive samples. All histopathological and Gram stain-positive tissues tested positive for fast urease. The differences in patients can be attributed to antibiotics use and other indications, including respiratory tract infections and dual resistance patterns, which carry a high risk for first-line therapy failure.

Five of the 29 *H. pylori*-positive PCR cases had mutations in the 23S rRNA gene at position 2243, indicating a clarithromycin resistance rate of 17.24%. Fontana et al., reported similar findings, in which point mutations at positions 2142 and 2143 were not discovered, but a T2717C point mutation was reported.[19] A study conducted by De Francesco et al.,[20] concluded that the antibiotic resistance rate of *H. pylori* was 17.2% for clarithromycin, which aligns with our research. However, the clarithromycin resistance rate varies globally, ranging from 49% in Spain to 1% in the Netherlands. While this might offer some insight into our observations, it also suggests that in cases where patients have mixed infections and receive antibiotic treatment, the naturally selected resistant strains could exacerbate the issue of drug resistance.

Interestingly, the T2243C mutation was found in all clarithromycin-resistant isolates in our region. While this study implies that T2243C plays a significant role in clarithromycin resistance, other mutations could be at work. In their investigation, Khademi et al.,[21] discovered similar mutations in the 23S rRNA gene at position T2243C.

Limitations of study

The study's limitations include a small sample size (105 patients) and single-center design, potentially limiting generalizability. Lack of long-term follow-up and absence of a control group may hinder comprehensive

insights into *H. pylori* infection's impact on disease progression. Diagnostic methods used, though varied, might have inherent limitations affecting accuracy. To strengthen findings, future research should consider larger samples, multi-center studies, longer follow-ups, and the incorporation of updated diagnostic methods.

CONCLUSION

H. pylori was found in 27.67% of individuals with acid-peptic illness in this location. An in-house RUT was utilized to detect *H. pylori* infection, which is a cheap, simple, and quick method. Although culture is regarded as the gold standard, it is difficult, costly, necessitates an enriched transport medium, and yields delayed results. *H. pylori* PCR is a highly effective, high-detection approach. As a result, combining RUT and PCR is a valuable diagnostic strategy. Fluoroquinolone-containing second-line drugs, such as levofloxacin and moxifloxacin, are utilized as alternatives to clarithromycin in drug-resistant individuals.

In conclusion, we discovered a new point mutation, T2243C, in the *H. pylori* 23S rRNA gene that contributes to clarithromycin resistance in our isolates. This mutation pattern shows geographical variations. Further research incorporating the full 23S rRNA gene is required to uncover other possible point mutations.

ACKNOWLEDGMENT

We sincerely thank the patients, medical staff, and Saveetha Medical College and Hospitals for supporting this study. Special thanks to the Department of Microbiology and the Human Institutional Ethical Committee.

REFERENCES

1. Hunt RH, Xiao SD, Megraud F, Leon-Barua R, Bazzoli F, Van Der Merwe S, et al. *Helicobacter pylori* in developing countries. World Gastroenterology Organisation Global Guideline. J Gastrointestin Liver Dis. 2011;20(3):299-304.
2. Björkholm B, Sjolund BM, Falk PG, Berg OG, Engstrand L and Andersson DI. Mutation frequency and biological cost of antibiotic resistance in *Helicobacter pylori*. Proc Natl Acad Sci U S A. 2001;98(25):14607-14612.
   https://doi.org/10.1073/pnas.241517298
3. Mbulaiteye SM, Hisada M and El-Omar EM. *Helicobacter pylori* associated global gastric cancer burden. Front Biosci (Landmark Ed). 2009;14(4):1490-1504.
   https://doi.org/10.2741/3320
4. Goodwin CS, McCulloch RK, Armstrong JA and Wee SH. Unusual cellular fatty acids and distinctive ultrastructure in a new spiral bacterium (*Campylobacter pyloridis*) from the human gastric mucosa. J Med Microbiol. 1985;19(2):257-267.
   https://doi.org/10.1099/00222615-19-2-257
5. Gerrits MM, Van Vliet AH, Kuipers EJ and Kusters JG. *Helicobacter pylori* and antimicrobial resistance: Molecular mechanisms and clinical implications. Lancet Infect Dis. 2006;6(11):699-709.
   https://doi.org/10.1016/S1473-3099(06)70627-2
6. Park SA, Ko A and Lee NG. Stimulation of growth of the human gastric pathogen *Helicobacter pylori* by atmospheric level of oxygen under high carbon dioxide tension. BMC Microbiol. 2011;11:96.
   https://doi.org/10.1186/1471-2180-11-96
7. Guarner J, Kalach N, Elitsur Y and Koletzko S. *Helicobacter pylori* diagnostic tests in children: Review of the literature from 1999 to 2009. Eur J Pediatr. 2010;169(1):15-25.
   https://doi.org/10.1007/s00431-009-1033-x
8. Ueda J, Okuda M, Nishiyama T, Lin Y, Fukuda Y and Kikuchi S. Diagnostic accuracy of the E-plate serum antibody test kit in detecting *Helicobacter pylori* infection among Japanese children. J Epidemiol. 2014;24(1):47-51.
   https://doi.org/10.2188/jea.je20130078
9. Velayos B, Fernández-Salazar L, Pons-Renedo F, Muñoz MF, Almaraz A, Aller R, et al. Accuracy of urea breath test performed immediately after emergency endoscopy in peptic ulcer bleeding. Dig Dis Sci. 2012;57(7):1880-1886.
   https://doi.org/10.1007/s10620-012-2096-5
10. Subbukesavaraja V and Balan K. Comparative study of invasive methods for diagnosis of *Helicobacter pylori* in humans. Int J Curr Microbiol Appl Sci. 2013;2(7):63-68.
11. Khalifehgholi M, Shamsipour F, Ajhdarkosh H, Ebrahimi Daryani N, Pourmand MR, Hosseini M, et al. Comparison of five diagnostic methods for *Helicobacter pylori*. Iran J Microbiol. 2013;5(4):396-401.
12. Sivaprakash R, Rao U. Indigenous, simple, sensitive and cost effective urease test in the diagnosis of *H. pylori* for the developing world. Indian J Med Microbiol. 1994;12:111–5.
13. Archana D, Jaysheree P and Athanikar VS. *H. pylori* associated gastritis. J Clin Diagn Res. 2012;6(2):211-214.
14. Reddy BS, Venkateswarlu, Jyothi BN and Devi R. Role of *H. Pylori* in gastroduodenal diseases. J Evol Med Dent Sci. 2015;4(4):581-586.
   https://doi.org/10.14260/jemds/2015/86
15. Chattopadhyay S, Patra R, Ramamurthy T, Chowdhury A, SantraA, Dhali GK, et al. Multiplex PCR assay for rapid detection and genotyping of *Helicobacter pylori* directly from biopsy specimens. J Clin Microbiol. 2004;42(6):2821-2824.
   https://doi.org/10.1128/JCM.42.6.2821-2824.2004
16. Arora U, Aggarwal A and Singh K. Comparative evaluation of conventional methods and ELISA based IgG antibodies detection for diagnosis of *Helicobacter pylori* infection in cases of dyspepsia. Indian J Med Microbiol. 2003;21(1):46-48.
17. Shrestha R, Koirala K, Raj KC and Batajoo KH. *Helicobacter pylori* infection among patients with upper gastrointestinal symptoms: Prevalence and relation to endoscopy diagnosis and histopathology. J Family Med Prim Care. 2014;3(2):154-158.
   https://doi.org/10.4103/2249-4863.137663
18. Clayton CL, Kleanthous H, Coates PJ, Morgan DD and Tabaqchali S. Sensitive detection of *Helicobacter pylori* by using polymerase chain reaction. J Clin Microbiol. 1992;30(1):192-200.
   https://doi.org/10.1128/jcm.30.1.192-200.1992
19. Fontana C, Favaro M, Minelli S, Criscuolo AA, Pietroiusti A, Galante A, et al. New site of modification of 23S rRNA associated with clarithromycin resistance of *Helicobacter pylori* clinical isolates. Antimicrob Agents Chemother. 2002;46(12):3765-3769.

https://doi.org/10.1128/AAC.46.12.3765-3769.2002

20. De Francesco V, Giorgio F, Hassan C, Manes G, Vannella L, Panella C, et al. Worldwide *H. pylori* antibiotic resistance: A systematic review. J Gastrointestin Liver Dis. 2010;19(4):409-414.
21. Khademi F, Faghri J, Moghim S, Esfahani BN, Fazeli H, Poursina F, et al. The study of mutation in 23S rRNA resistance gene of *Helicobacter pylori* to clarithromycin in patients with gastrointestinal disorders in Isfahan-Iran. Adv Biomed Res. 2014;3:98.

https://doi.org/10.4103/2277-9175.129368

Authors Contribution:
MER – Manuscript preparation, performed the procedure; **PS-** Review Manuscript, **RSAG –** Protocol review, Review Manuscript; **RDS –** Literature review, Data collection, Data analysis; and **NMR –** Editing Manuscript.

Work attributed to:
Department of Microbiology, Saveetha Medical College and Hospital, Chennai, Tamil Nadu, India.

Orcid ID:
Dr. Palaneswamy Savetha - https://orcid.org/0000-0002-4908-3092
Dr. Rajesh Samuel Ajit George - https://orcid.org/0000-0002-3173-3296
Dr. Ravin Devasir Sathyaseelan - https://orcid.org/0000-0003-2381-2098
Dr. Malini Evangeline Rose C - https://orcid.org/0000-0003-3035-9201
Dr. Noor Mohamed Rasik B - https://orcid.org/0009-0008-8458-7350

Source of Support: Nil, **Conflicts of Interest:** None declared.