



## DIAGNOSIS OF HELICOBACTER PYLORI AMONG GASTROINTESTINAL PATIENTS IN WAIST PROVINCE DURING 2022-2023

Zahra Ali Hussein<sup>1</sup>,

Khairi Jameell Al-Rubay<sup>2</sup>

<sup>1,2</sup>Department of Biology, College of Science, University of Wasit

Corresponding author: khairi2009jamel@gmail.com

### Abstract

**Background:** Helicobacter pylori, also known as H. pylori infection, is a growing global health concern and affects nearly 50% of the world's population. This persistent Infection remains a significant public health challenge. The objectives of this study are to identify the presence of Helicobacter pylori in various clinical cases of gastrointestinal disorders by using several biochemical tests and non-invasive procedures.

**Material and method:** This study was conducted in Waist Governorate and included 119 patients. Their ages ranged between 12 and 62 years old, and they were males and females. Blood specimens were obtained from patients in Al- Karama Teaching Hospital. Saliva and some blood specimens were obtained from the outpatient clinic. Based on the clinical Diagnosis according to antibody, stool antigen, and urea breath test to detect H. pylori infection

**Results:** the study found the gastroenteritis patients according to type H. Pylori test with the highest per cent 74.60% positive to serum antibody, and negative 57.60% regarding stool antigen, and highest negative 64.40% to urea breath test. The findings reveal significant relationships between different age groups and the levels of Serum antibody ( $p = .016$ ), Stool antigen ( $p = .000$ ), and Urea breath test ( $p = .008$ ). The study found high sensitivity and high specificity in the urea breath test at AUC (0.936) SD error (0.025) and stool antigen at AUC 0.875, SD error 0.052 with P.value  $< 0.05$  compared with serum antibody at AUC 0.741, SD error 0.08 with P.value 0.004

**conclusion:** The study indicted significant increase of H. Pylori infection with increase age of gastrointestinal patients of while observed no difference between males and females about these Infection, the urea breath test has high sensitive and specific for detecting H. Pylori infection than other non-invasive test.

**Keywords:** Helicobacter pylori, Diagnosis, gastrointestinal Infection



## Introduction

The *Helicobacter pylori* (*H. pylori*), previously called *Campylobacter pylori*, is spiral-shaped and Gram-negative. It thrives in environments with low oxygen levels and exhibits fine aerobic properties. It is common in the stomach area of about 50% of the world's population. *Helicobacter pylori* (*H. pylori*) is mainly transmitted by person-to-person contact and is usually acquired early in life [1-2]. It is common in people with chronic gastritis, which usually has no symptoms in about 85% of cases. However, it can also lead to more severe disorders such as peptic ulcers or stomach adenocarcinoma. The latter is particularly worrisome as it is responsible for many deaths worldwide, with an estimated annual death toll of over 800,000 people. According to the previous source [2]. *Helicobacter pylori* (*H. pylori*) is an essential causative agent in many gastrointestinal disorders, including gastric and duodenal ulcers, gastritis, and some subtypes of stomach cancer, such as adenocarcinoma and mucosa-associated lymphoid tissue carcinoma (MALT). The inputs submitted by the user are in the form of a list. *H. pylori* infection is highly prevalent, ranging from 30% to 50% in rich countries to 70% to 90% in poor countries [3]. The main methods of transmission for *H. pylori* are the transfer of the bacterium through the oral-faecal and oral-oral routes, necessitating intimate interpersonal interaction. *Helicobacter pylori* strains are frequently detected in tissue samples obtained from gastric biopsies. However, it is worth noting that these substances can also be identified in saliva, gastric reflux fluid, diarrheal samples, and vomitus.

## Materials and methods

This study was conducted in waist Governorate and included 119 patients. Their ages ranged between 12 and 62 years old, and they were males and females. Blood specimens were obtained from patients in Al-Karama Teaching Hospital. Saliva and some blood specimens were obtained from the outpatient clinic Based on the clinical Diagnosis. The study extends from October 2022 to March 2023. The specialist doctor diagnosed patients with gastroenteritis. Demographic data was collected for patients, such as age and gender.

### Specimens collection

Specimens were collected from the Consultant Internal Medicine and the Division of Gastroenterology at Al-Karama Teaching Hospital and outpatient clinic for both Sexes, as follows:

1- Blood specimens were collected from patients suffering from gastroenteritis; 3–5 ml of the patient's blood was taken and placed in a tube. The total number of specimens was 119 blood specimens from healthy people as a control group.





2- Saliva specimens were collected from the outpatient clinic.

#### 2.2.4. Specimens preservation

1- The blood serum specimens were placed in the Eppendorf tube and kept in deep freezing (-70 °C).

2- Saliva specimens were placed in disposable containers and preserved by deep freezing.

#### 2.2.5. Samples examination

##### 2.2.5.1 Urea Breath Test (UBT)

The participants were given explicit instructions to ingest a capsule containing urea marked with radioactive material. This ingestion was to occur precisely two hours after consuming a meal and before administering any antibiotic treatment. The YHO4E H. pylori test system (Figure 2.1) was employed to detect <sup>14</sup>C labelled urea. In the case of H. pylori infection, the urea in the test is metabolized, producing isotope-labelled carbon dioxide. This carbon dioxide is then exhaled by the patient and subsequently identified by the equipment. The methodology was executed per the manufacturer's requirements stipulated by Headway Company, China.

##### 2.2.5.2 Serum antibody test for Helicobacter pylori detection

The participants had phlebotomy, during which 3-5 mL of blood was extracted and placed in a gel tube. The blood samples were then allowed to reach room temperature, facilitating the formation of a blood clot. Following the sample centrifugation at a force of 4000 times the acceleration due to gravity for a duration ranging from 5 to 10 minutes, the serum was successfully separated, rendering the specimen prepared for subsequent analysis. The internal packing remained unopened until the moment of its intended use.

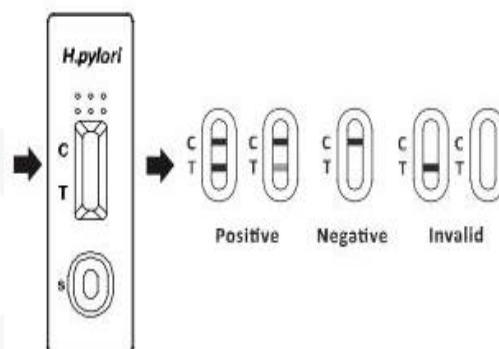


Figure (1.2): Assay Procedure of H. pylori rapid test



## Statistical analysis

The data analysis was performed utilizing the Statistical Package for Social Sciences (SPSS, United States) Statistics software, version 26. The data analysis encompassed the computation of diverse statistical ratios, such as percentages, means, and standard deviations, and the execution of a chi-square test. Statistical significance was assessed by employing a threshold of p-values below 0.05.

## Results

Table 4.1 Descriptive characteristic of the study subject

N	Parameter		Frequency	Percent
1	Age group	15-30	61	51.7%
		31-45	33	28.0%
		46->60	24	20.3%
		mean+SD	33.22+13.76	min-mix(15-71)
2	sex	Female	68	57.6%
		Male	50	42.4%
3	serum antibody	Positive	88	74.6%
		Negative	30	25.4%
4	stool antigen	Positive	50	42.4%
		Negative	68	57.6%
5	urea breath test	Positive	42	35.6%
		Negative	76	64.4%

The results reveal notable participant characteristics. The average age of the study sample was 33.22 years (Standard Deviation = 13.76). The age range of 15-30 years constituted the most significant portion, accounting for 51.7% of the participants. In terms of gender distribution, females made up more than half of the participants (57.6%), while males comprised the remaining 42.4%. Regarding serum antibody levels, approximately one-third of the study sample tested positive (74.6%). In the context of stool antigen analysis, negative results were predominant, representing 57.6% of the cases. Lastly, the urea breath test indicated adverse outcomes for 64.4% of the participants. as presented in Table 4.1

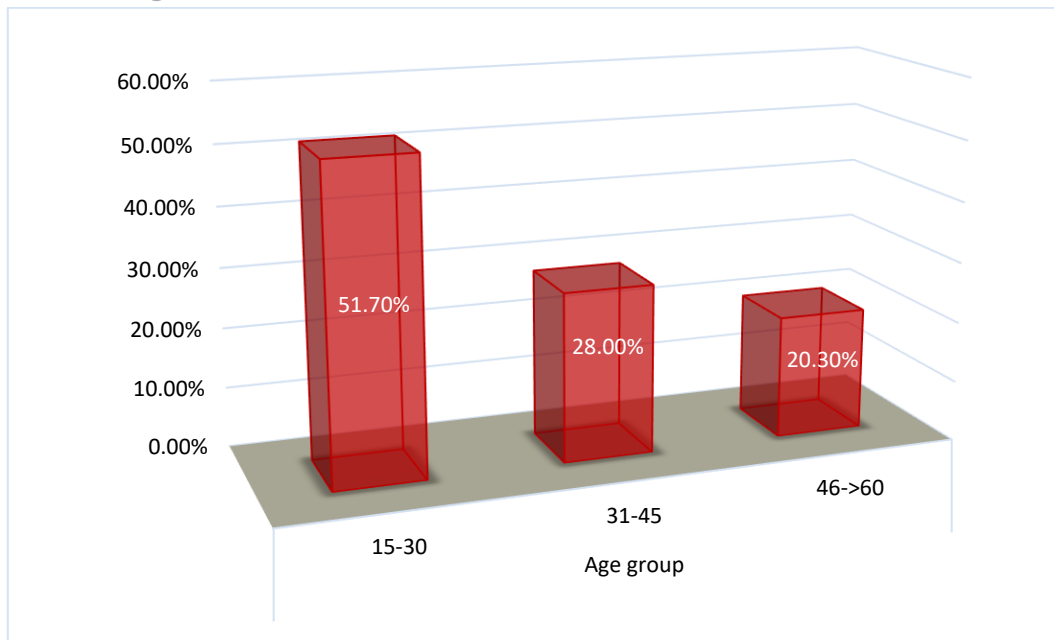


Figure 1.3 Distribution of gastroenteritis patients according to age group

The results reveal notable participant characteristics. The average age of the study sample was 33.22 years (Standard Deviation = 13.76). The age range of 15-30 years constituted the most considerable portion, accounting for 51.7% of the participants. as shown in figure 1.3

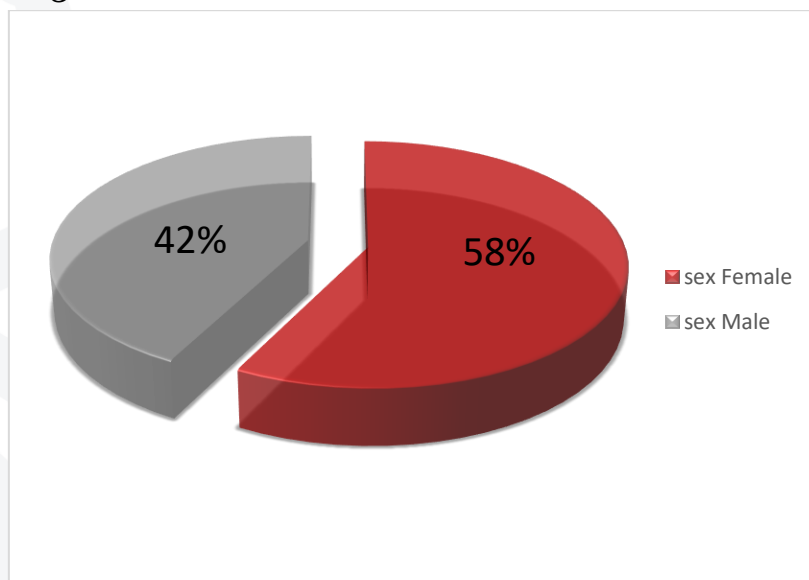


Figure 1.4 Distribution of gastroenteritis patients according to sex

In terms of gender distribution, females made up more than half of the participants (57.6%), while males comprised the remaining 42.4%.

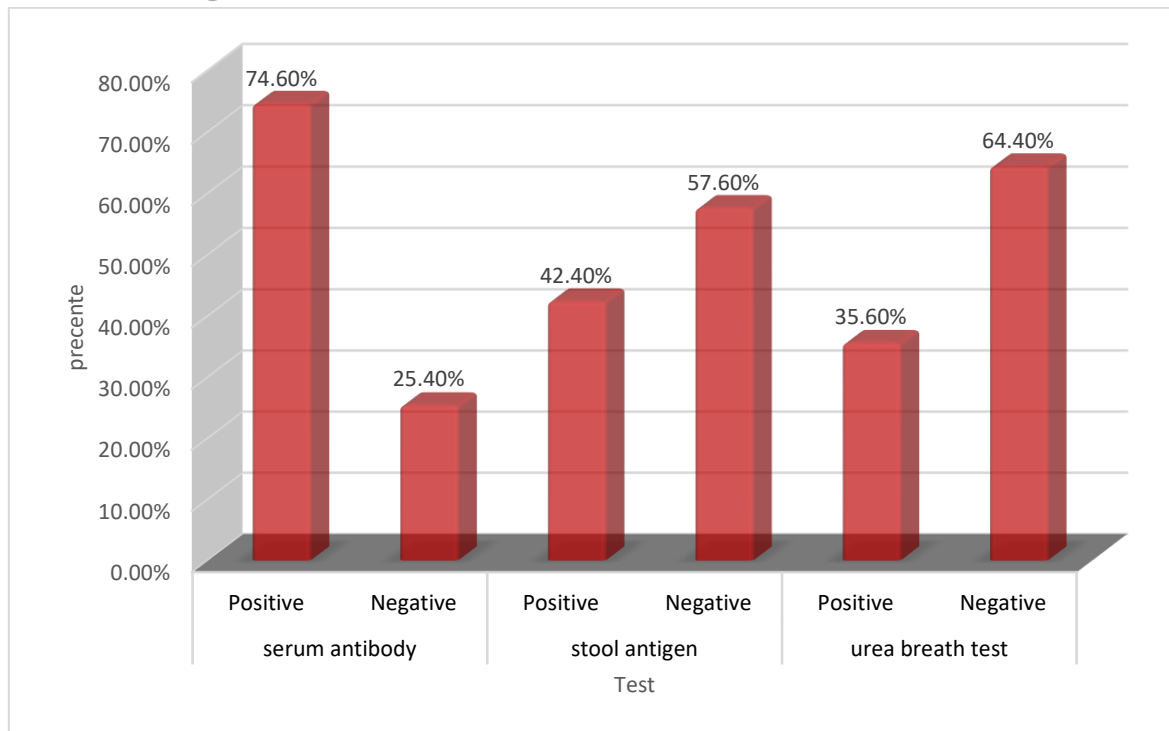


Figure 1.5 Distribution of gastroenteritis patients according to type H.pylori test

The results in Figure 1.5 showed that the distribution of gastroenteritis patients according to type H.pylori test was the highest percent 74.60% positive to serum antibody, and negative 57.60% regarding to stool antigen, and highest negative 64.40% to urea breath test.

Table 4.2 evaluation of type of H. pylori tests according to age group

N	Test		Age group			X <sup>2</sup>	Sig	
			15-30	31-45	46->60			
1	Serum antibody	Positive	Count	39	27	22	8.254 <sup>a</sup>	0.016
			%	44.3%	30.7%	25.0%		
		Negative	Count	22	6	2		
			%	73.3%	20.0%	6.7%		
2	Stool antigen	Positive	Count	16	24	10	18.967 <sup>a</sup>	0.000
			%	32.0%	48.0%	20.0%		
		Negative	Count	45	9	14		
			%	66.2%	13.2%	20.6%		
3	Urea breath test	Positive	Count	16	19	7	9.722 <sup>a</sup>	0.008
			%	38.1%	45.2%	16.7%		
		Negative	Count	45	14	17		
			%	59.2%	18.4%	22.4%		



The findings reveal noteworthy relationships between different age groups and the levels of Serum antibody ( $p = .016$ ), Stool antigen ( $p = .000$ ), and Urea breath test ( $p = .008$ ).

Table 4.3 evaluation of type of *H. pylori* tests according to sex

N	Test	sex		$\chi^2$	odds ratio	sig		
		female	male					
1	serum antibody	positive	Count	54	34	1.979 <sup>a</sup>	0.551	0.159
			%	61.4%	38.6%			
		negative	Count	14	16			
			%	46.7%	53.3%			
2	stool antigen	positive	Count	28	22	.094 <sup>a</sup>	1.122	0.759
			%	56.0%	44.0%			
		negative	Count	40	28			
			%	58.8%	41.2%			
3	urea breath test	positive	Count	25	17	.096 <sup>a</sup>	0.886	0.757
			%	59.5%	40.5%			
		negative	Count	43	33			
			%	56.6%	43.4%			

The results indicate that there was no observed relationship between sex and the levels of Serum antibody ( $p = 0.159$ ), Stool antigen ( $p = 0.759$ ), and Urea breath test ( $p = 0.757$ ).

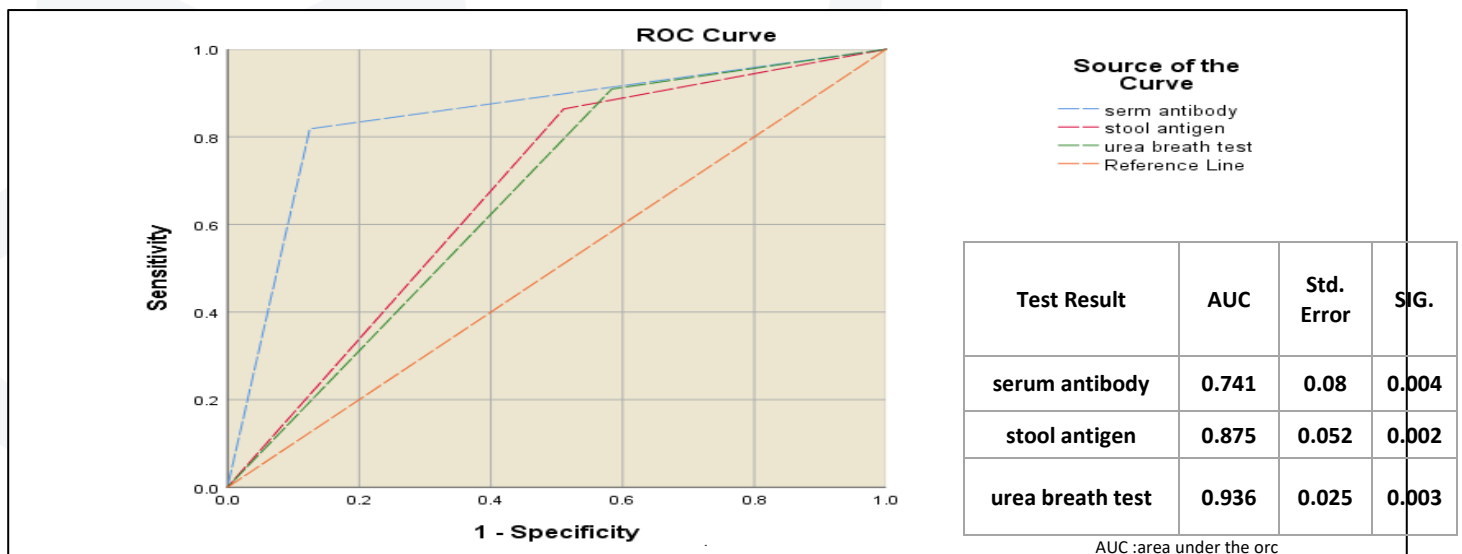


Figure 1.6 The sensitivity and specificity of the *H. Pylori* test



Figure 1.6 shows the sensitivity and specificity of serology, serum antibody, stool antigen, and urea breath test. The study found high sensitivity and high specific in urea breath test at AUC (0.936) SD error (0.025) and stool antigen at AUC 0.875, SD error 0.052 with P.value <0.05 compared with serum antibody at AUC 0.741, SD error 0.08 with P.value 0.004

#### Discussion

Half of the world's population has *H. pylori*, a traumatic illness with an unfortunate 90% estimated prevalence in underdeveloped nations [13-16]. Multiple epidemiological studies have pointed to substantial variations in global frequency, emphasizing socioeconomic factors such as overcrowding, poor sanitation, cleanliness, and patient behaviour [17-19]. Our findings are consistent with those of a study recently conducted. Researchers found different results in their study, which also found that 81.95% of infected patients were detected using serum antibody tests [20], in which most patient specimens (55.6% on average) tested positive for serological markers. Individual differences in immunological response may account for the discrepancy in study outcomes [21]. A serum antibody test can be completed quickly and easily. It is also a quick and accurate test for detecting *H. pylori* antibodies in patient serum. It can be utilized for routine testing in labs and the field without requiring particular instruments or gadgets [22]. These findings closely align with the results of the current study. The study by a researcher found that (33.3%) of patient specimens showed positive results for the stool antigen test. These findings differed from the results of a previous study by She X et al. titled "Prevalence of Helicobacter Pylori Infection Concerning Age, Socioeconomic Status, and Dietary Habits in a Population-based Study in China." The study conducted in China revealed a correlation between age and the prevalence of *H. pylori* infection. [23]

The findings indicated that the infection rate increased progressively with age, starting from 32.8% in individuals aged 18-29 and reaching 57.1% in those aged 60—the study conducted by Wang et al. The study was a comprehensive analysis of various studies in China, utilizing systematic review and meta-analysis methodologies. [24]

According to the authors, there has been a decline in the prevalence of *H. pylori* infection among children and adolescents in China in recent years. However, the infection rate remains higher in China compared to developed countries. "Helicobacter pylori infection in older people: A systematic review and meta-analysis" by Gong et al. This study was a systematic review and meta-analysis of studies conducted in older people. The authors found that the prevalence of *H. pylori* infection is higher in older than younger people. [25]







"The relationship between age and Helicobacter pylori infection: A systematic review and meta-analysis" (2022) by Ren et al. This study was a systematic review and meta-analysis of studies conducted in different populations worldwide. The authors found that the prevalence of H. pylori infection increases with age. However, the rate of increase varies between populations. [26] Zhang et al. This study find that the prevalence of H. pylori infection increased with age, from 43.5% in people aged 18-29 to 57.1% in people aged 60 years or older. The authors also found that the severity of H. pylori infection is higher in older people. [27] These studies suggest that the prevalence of H. pylori infection increases with age. Due to several factors, including increased exposure to the bacteria, decreased gastric acidity, and decreased immune function. The relationship between sex and Helicobacter pylori Infection: A systematic review and meta-analysis" by Huang et al. This study was a systematic review and meta-analysis of studies conducted in different populations worldwide. The authors found a weak association between sex and H. pylori infection, with men slightly more likely to be infected than women.[28]"The prevalence of Helicobacter pylori Infection in children and adolescents in China: A systematic review and meta-analysis" (2023) by Wang et al. This study was a systematic review and meta-analysis of studies conducted in China. The authors found that the prevalence of H. pylori infection in children and adolescents in China is higher in boys than in girls.[29]"Helicobacter pylori infection in older people: A systematic review and meta-analysis" (2022) by Buzás et al. This study was A Narrative Review of studies conducted in older people. The authors found that the prevalence of H. pylori infection is higher in men than women.[30]

The relationship between sex and Helicobacter pylori infection by Advanced Sensitivity and Discrimination Tools. Diagnostics by Cardos et al. This study was a systematic review and meta-analysis of studies conducted in children. The authors found a weak association between sex and H. pylori infection in children, with boys slightly more likely to be infected than girls. These previous studies suggest a weak association between sex and H. pylori infection, with men slightly more likely to be infected than women. However, the reasons for this association are not fully understood. [31]The sensitivity and specificity of stool antigen tests for diagnosing H. pylori appear to differ. In our study, the HpSA test demonstrated a sensitivity of 70%, which aligns with the results of the Megraud study. However, it is worth noting that the test's specificity in our study was lower at 77%, contrasting with the findings of Megraud. Furthermore, it is worth noting that the HpSA test yielded more accurate results in children aged six years and below, as opposed to older children. This discrepancy can be attributed primarily to the higher incidence of false-negative





outcomes observed in the older age group.[32]The performance of the HpStar stool antigen kit was excellent across all age groups tested. However, it is worth noting that the specificity of the kit was slightly lower in younger children, although this difference was statistically significant. Our study's results show a favourable comparison with the study conducted by Sykora et al. In their study, the HpStar test demonstrated a sensitivity of 96.1% and specificity of 98.5%. The positive and negative predictive values were also 96.1% and 98.5% respectively [33] The discrepancy in outcomes observed between the HpSA test and the HpStar test is uncertain. However, it could be attributed to the use of polyclonal antibodies in the HpSA assay. In contrast, the HpStar test employs a monoclonal antibody for antigen capture. [34-36]

#### Conclusions and recommendations

The study indicated a significant increase in H. Pylori infection with the increased age of gastrointestinal patients while observing no difference between males and females about these infections; the urea breath test is more sensitive and specific for detecting H. Pylori infection than another non-invasive test; the study recommended that early detection through regular examination of H. Pylori in elderly age

#### Reference

1. Reshetnyak VI, Burmistrov AI, Maev IV. Helicobacter pylori: Commensal, symbiont or pathogen?. World journal of gastroenterology. 2021 Feb 2;27(7):545.
2. Kiran N, Kashi M, Khan S. Association of Collagenous Gastritis With Helicobacter pylori Infection. Cureus. 2023 Jul 20;15(7).
3. Khoder G, Muhammad JS, Mahmoud I, Soliman SS, Burucoa C. Prevalence of Helicobacter pylori and its associated factors among healthy asymptomatic residents in the United Arab Emirates. Pathogens. 2019 Apr 1;8(2):44.
4. White, J. R., Winter, J. A., & Robinson, K. (2015). Differential inflammatory response to Helicobacter pylori infection: etiology and clinical outcomes. *Journal of inflammation research*, 137-147.
5. Kismat S, Tanni NN, Akhtar R, Roy CK, Rahman MM, Molla MM, Anwar S, Ahmed S. Diagnosis and Comparison of Three Invasive Detection Methods for Helicobacter pylori Infection. Microbiology Insights. 2022 Oct;15:11786361221133947.
6. Hussein RA, Al-Ouqaili MT, Majeed YH. Detection of Helicobacter Pylori infection by invasive and noninvasive techniques in patients with gastrointestinal diseases from Iraq: A validation study. Plos one. 2021 Aug 23;16(8):e0256393.
7. Ansari S, Yamaoka Y. Helicobacter pylori virulence factors exploiting gastric colonisation and its pathogenicity. Toxins. 2019 Nov 19;11(11):677.





8. Oleastro M, Ménard A. The role of *Helicobacter pylori* outer membrane proteins in adherence and pathogenesis. *Biology*. 2013 Aug 27;2(3):1110-34.
9. Kao CY, Sheu BS, Wu JJ. *Helicobacter pylori* infection: An overview of bacterial virulence factors and pathogenesis. *Biomedical journal*. 2016 Feb 1;39(1):14-23.
10. Zhang Z, Liu F, Ai F, Chen X, Liu R, Zhang C, Fang N, Fu T, Wang X, Tang A. The efficacy and mechanism of vonoprazan-containing triple therapy in the eradication of *Helicobacter pylori*. *Frontiers in Pharmacology*. 2023 May 5;14:1143969.
11. Tilahun M, Gedefie A, Belayhun C, Sahle Z, Abera A. *Helicobacter pylori* pathogenicity islands and *Giardia lamblia* cysteine proteases in role of coinfection and pathogenesis. *Infection and Drug Resistance*. 2022 Jan 1:21-34.
12. Fu HW, Lai YC. The Role of *Helicobacter pylori* Neutrophil-Activating Protein in the Pathogenesis of *H. pylori* and Beyond: From a Virulence Factor to Therapeutic Targets and Therapeutic Agents. *International Journal of Molecular Sciences*. 2022 Dec 21;24(1):91.
13. Raj P, Thompson JF, Pan DH. *Helicobacter pylori* serology testing is a useful diagnostic screening tool for symptomatic inner city children. *Acta paediatrica*. 2017 Mar;106(3):470-7.
14. Salih BA. *Helicobacter pylori* infection in developing countries: the burden for how long? *Saudi J Gastroenterol*. 2009;15(3):201-7.
15. . Pk B. Epidemiological features of *Helicobacter pylori* infection in developing countries. *Clin Infect Dis*. 1997;25(5):973-8.
16. Archampong TNA, Asmah RH, Wiredu EK, Gyasi RK, Nkrumah KN, Rajakumar K. Epidemiology of *Helicobacter pylori* infection in dyspeptic Ghanaian patients. *Pan Afr Med J*. 2015;20:1-9
17. Rosenstock SJ, Andersen LP, Rosenstock CV, Bonnevie O, Jørgensen T. Socioeconomic factors in *Helicobacter pylori* infection among Danish adults. *Am J Public Health*. 1996;86(11):1539-44.
18. Chen HL, Chen MJ, Shih SC, Wang HY, Lin IT, Bair MJ. Socioeconomic status, personal habits, and prevalence of *Helicobacter pylori* infection in the inhabitants of Lanyu. *J Formos Med Assoc*. 2014;113(5):278-83. <https://doi.org/10.1016/j.jfma.2013.11.013>.
19. Ansari S, Gautam R, Nepal HP, Subedi SN, Shrestha S, Mandal F, et al. *Helicobacter pylori* colonisation in Nepal; Assessment of prevalence and potential risk factors in a hospital-based patient cohort microbiology. *BMC Res Notes*. 2016;9(1):4-9.



20. Luo JC. Noninvasive diagnostic methods for Helicobacter pylori infection. Journal of the Chinese Medical Association. 2015 Feb 1;78(2):83-4.
21. Patel SK, Pratap CB, Jain AK, Gulati AK, Nath G. Diagnosis of Helicobacter pylori: what should be the gold standard?. World journal of gastroenterology: WJG. 2014 Sep 9;20(36):12847.
22. Opekun AR, Zierold C, Rode A, Blocki FA, Fiorini G, Saracino IM, Vaira D, Sutton FM. Clinical performance of the automated LIAISON® meridian H. pylori SA stool antigen test. BioMed research international. 2020 Mar 19;2020.
23. She X, Zhao J, Cheng S, Shi H, Dong L, Zhao P. Prevalence of and risk factors for Helicobacter pylori infection in rural areas of Northwest China: A cross-sectional study in two villages of Yan'an city. Clinical Epidemiology and Global Health. 2023 May 1;21:101294.
24. Araújo GR, Marques HS, Santos ML, da Silva FA, da Brito BB, Santos GL, de Melo FF. Helicobacter pylori infection: How does age influence the inflammatory pattern? World Journal of Gastroenterology. 2022 Jan 1;28(4):402.
25. Wang YK, Kuo FC, Liu CJ, Wu MC, Shih HY, Wang SS, Wu JY, Kuo CH, Huang YK, Wu DC. Diagnosis of Helicobacter pylori infection: Current options and developments. World Journal of Gastroenterology: WJG. 2015 Oct 10;21(40):11221.
26. Ren S, Cai P, Liu Y, Wang T, Zhang Y, Li Q, Gu Y, Wei L, Yan C, Jin G. Prevalence of Helicobacter pylori infection in China: A systematic review and meta-analysis. Journal of gastroenterology and hepatology. 2022 Mar;37(3):464-70.
27. Che, T.H., Nguyen, T.C., Vu, V.N.T., Nguyen, H.T., Hoang, D.T.P., Ngo, X.M., Truong, D.Q., Bontems, P., Robert, A. and Nguyen, P.N.V., 2023. Factors Associated With Helicobacter Pylori Infection Among School-Aged Children From a High Prevalence Area in Vietnam. *International journal of public health*, 68, p.1605908.
28. Hathroubi S, Servetas SL, Windham I, Merrell DS, Ottemann KM. Helicobacter pylori biofilm formation and its potential role in pathogenesis. Microbiology and Molecular Biology Reviews. 2018 Jun;82(2):10-128.
29. Gong H, Xu HM, Zhang DK. Focusing on Helicobacter pylori infection in the elderly. Frontiers in Cellular and Infection Microbiology. 2023 Mar 10;13:1121947.
30. Buzás GM, Birinyi P. Newer, Older, and Alternative Agents for the Eradication of Helicobacter pylori Infection: A Narrative Review. Antibiotics. 2023 May 23;12(6):946.
31. Cardos AI, Maghiar A, Zaha DC, Pop O, Fritea L, Miere F, Cavalu S. Evolution of Diagnostic Methods for Helicobacter pylori Infections: From Traditional Tests to





- High Technology, Advanced Sensitivity and Discrimination Tools. *Diagnostics*. 2022 Feb 16;12(2):508
32. Gisbert JP, Pajares JM. Diagnosis of *Helicobacter pylori* infection by stool antigen determination: a systematic review. *The American journal of gastroenterology*. 2001 Oct 1;96(10):2829-38.
33. Best, L. M., Takwoingi, Y., Siddique, S., Selladurai, A., Gandhi, A., Low, B., Yaghoobi, M., & Gurusamy, K. S. (2018). Non-invasive diagnostic tests for *Helicobacter pylori* infection. *The Cochrane database of systematic reviews*, 3(3), CD012080. <https://doi.org/10.1002/14651858.CD012080.pub2>
34. Ansari, S., & Yamaoka, Y. (2022). *Helicobacter pylori* Infection, Its Laboratory Diagnosis, and Antimicrobial Resistance: a Perspective of Clinical Relevance. *Clinical microbiology reviews*, 35(3), e0025821. <https://doi.org/10.1128/cmr.00258-21>
35. Elbehiry A, Marzouk E, Aldubaib M, Abalkhail A, Anagreyyah S, Anajirih N, Almuzaini AM, Rawway M, Alfadhel A, Draz A, Abu-Okail A. *Helicobacter pylori* infection: Current status and future prospects on diagnostic, therapeutic and control challenges. *Antibiotics*. 2023 Jan 17;12(2):191.
36. Huang J, Liu Z, Ma J, Liu J, Lv M, Wang F, Tang X. The association between *helicobacter pylori* seropositivity and bone mineral density in adults. *Mediators of Inflammation*. 2022 Apr 4;2022.

