

Original Research Article

ANTIGEN DETECTION OF THE PREVALENCE RATE OF *HELICOBACTER PYLORI* AMONGST PATIENTS PRESENTING WITH PRESUMPTIVE GASTRITIS IN RIVERS STATE UNIVERSITY TEACHING HOSPITAL PORT HARCOURT.

ABSTRACT

Background: Gastritis is an inflammation of the protective lining of the stomach which has been a global burden in the past decades. It can be caused by irritation, excessive alcohol use, chronic vomiting, stress, or the use of certain medications such as aspirin or other anti-inflammatory drugs. It may also be caused by *Helicobacter pylori*- a gram-negative, microaerophilic, spiral (helical) bacterium usually found in the stomach.

Methodology: A cross-sectional, hospital-based study aimed at determining the prevalence rate of *Helicobacter pylori* amongst patients presenting with presumptive gastritis was carried out at gastroenterology clinic of Rivers State University Teaching Hospital Port Harcourt. Two hundred and forty two (242) male and female participants were recruited randomly for the study, their stool samples were analyzed using On Site *H. Pylori* antigen (USA) and fecal occult blood (ROSTEC) Rapid Diagnostic Test Kits, while socio demographic characteristics and symptoms for gastritis were assessed with pretest questionnaires. Data generated from this study were statistically analyzed using MS Excel 2007 and represented in charts and tables.

Results: The prevalence rate of *H. Pylori* was found to be 55% in Rivers State University teaching Hospital (RSUTH) with a higher prevalence among females than males and an age dependent increase in prevalence but decline above 49years.

Conclusion: Thus, it is imperative for proactive actions to be taken in our homes, community and societies at large on public hygiene, food hygiene as well as public health and community sanitation. Proper education of citizens on the modes of transmission and prevention of *H. pylori* infection is also required to halt the trend of infection of this bacteria in our society.

Key words: Gastritis, *Helicobacter pylori*, occult blood, antigen

1. INTRODUCTION

Gastritis is an inflammation of the protective lining of the stomach [1]. It can be acute gastritis which involves sudden, severe inflammation or chronic gastritis which involves long-term inflammation that can last for years if it is left untreated [2]. Gastritis can be asymptomatic also but when symptoms are present, the most common is upper abdominal pain[3]. Other possible symptoms include nausea and vomiting, bloating, loss of appetite and heartburn. Complications may include stomach

bleeding, stomach ulcers, and stomach tumors [4 3]. Gastritis is believed to affect about half of people worldwide [5]. In 2013 there were approximately 90 million new cases of the condition[6] It can be caused by irritation due to excessive alcohol use, chronic vomiting, stress, or the use of certain medications such as aspirin or other anti-inflammatory drugs. It may also be caused by the bacteria *Helicobacter pylori* [7].

Helicobacter pylori, previously known as *Campylobacter pylori*, is a gram-negative, microaerophilic, spiral (helical) bacterium usually found in the stomach [8]. The bacterium was first identified in 1982 by Australian physicians; Barry Marshall and Robin Warren [9, 10]. *H. Pylori* has been associated with lymphomas of the mucosa-associated lymphoid tissue in the stomach, esophagus, colon, rectum, or tissues around the eye (called extranodal marginal zone B-cell lymphoma of the cited organ) [11, 12] and of lymphoid tissue in the stomach called diffuse large B-cell lymphoma[13]. *H. Pylori* infection is usually asymptomatic but sometimes causes gastritis (stomach inflammation) or ulcers of the stomach or first part of the small intestine. The infection is also associated with the development of certain cancers occurring in less than 20% of cases [14]. Many researchers have suggested that *H. Pylori* causes or prevents a wide range of other diseases however many of these relationships remain controversial [15, 16].

In 2015, it was estimated that over 50% of the world's population had *H. Pylori* in their upper gastrointestinal tracts [17] with this infection (or colonization) being more common in developing countries [18]. In the developed world, the prevalence rates of *H. Pylori* vary from 1.2% to 12.2%, while in developing countries, the prevalence rates are much higher, about 70% to 90% of the populations harbor *H. Pylori* [19, 20]. Studies by Bashir and Ali,[21] in Kano (Nigeria) reported an *H. Pylori* prevalence of 81%, Malu *et al.*, [22] in Jos (Nigeria), found a prevalence of 87%, Ayodele *et al.*,

[23] reported a prevalence of 19.6% in University of Port Harcourt Teaching Hospital, Rivers State Nigeria, while Aboderin *et al.*, [24] reported 73% in South-West Nigeria. The prevalence of the infection varies according to different ages, socioeconomic strata and geographical regions[25, 26].

In developing countries the prevalence of *H. Pylori* is higher in children, due to lower socioeconomic status, poor hygiene, overpopulation and lack of safe drinking water, and in an older patient, the presentation of *H. Pylori* infection may be subtle or atypical, which may delay the diagnosis[27]. With advanced age (the Elderly), the increased presence of concomitant diseases and multidrug therapy, especially medications causing gastric mucosal damage and bleeding (e.g. Non-Steroidal Anti-Inflammatory Drugs (NSAID), bisphosphonates, antiplatelet drugs, warfarin), can lead to increased and severe complications of *H. Pylori* infection [28]. Infection with *H. Pylori* is characterized with these symptoms or patients may remain asymptomatic and its rate and complications are still increasing worldwide [29, 30], hence the need to evaluate the prevalence of *H. Pylori* among patients with gastritis attending the Gastrointestinal Tract Clinic in Rivers State University Teaching Hospital Port Harcourt, Nigeria.

2. MATERIALS AND METHOD

2.1 Experimental Design

The study is a cross-sectional, hospital-based study aimed at determining the prevalence of *Helicobacter pylori* amongst patients presenting with presumptive gastritis in Rivers State University Teaching Hospital Port Harcourt using antigen detection method.

2.2 Study Area

Patients attending the gastrointestinal clinic of the Rivers State University Teaching Hospital were recruited for this study. The Rivers State University Teaching Hospital is a hospital owned by the government of Rivers State and is located at 5-8 Harley Street, Old GRA Port Harcourt, Rivers State, Nigeria. Port Harcourt, the capital of Rivers State, is located on latitude 4.750N and longitude 7.000E and lies along Bonny River in the Niger Delta. Rivers State University Teaching Hospital has 375 licensed beds and 731 medical staff members. Its departments include Medicine, Paediatrics, Laboratories, Radiology, Family Medicine, Obstetrics and Gynaecology, Anaesthesia, Surgery, Pathology, Ophthalmology, Accident Centre and the Surgical/Medical Emergency. Some other departments are Pharmacy, Finance, Maintenance and General Administration.

2.3 Study Population

A total of 242 male and female patients attending the GIT clinic of Rivers State University Teaching Hospital from November 2021 to January 2022 were recruited for this study.

2.4 Sample Size Determination

Purposive sampling and randomized method were used in the selection of subjects for the antigen detection method, taking into consideration the prevalence of *H. Pylori* infection previously reported at University of Port Harcourt Teaching Hospital (UPTH) using antibody detection method by Ayodele *et al.*, [23]. The sample size was calculated using the Cochran's sample size formula as shown below [31]

$$N = \frac{Z^2 pq}{d^2}$$

Where N= the desired sample size

Z= the standard normal deviation set at 1.96 corresponding to 95% confidence level

p= the prevalence of population=19.6% or 0.196[23].

q=1-p

d=degree of accuracy desired at 0.05

Therefore

$$N = \frac{(1.96)^2 \times 0.196 \times (1 - 0.196)}{(0.05)^2}$$

N=242

2.5 Ethical Consideration/Informed Consent

Ethical clearance was obtained from the Rivers State Health Research Ethics committee and the Department of Medical Laboratory Science, Rivers State University, Port Harcourt. Signed informed consent was also obtained from the respective participants prior to enrolment. Data were obtained using questionnaires to establish the socio-demographic characteristics and presence of risk factors.

2.6 Eligibility Studies

2.6.1 Inclusion Criteria

1. Patients visiting the gastrointestinal tract clinic of the Rivers State University Teaching Hospital and with signs and symptoms of gastritis irrespective of their age, sex and ethnicity were included into this study.
2. Only those willing to provide at least oral consent were included in the study

2.6.2 Exclusion Criteria

3. Patients without signs of gastritis were excluded from this study
4. Patients who did not consent to enrolment in this study were also excluded

2.7 Stool Sample Collection

Stool samples were collected into sterile sample containers already labeled with patient's name. The samples were screened within 24 hours of collection using the *Helicobacter pylori* antigen and fecal occult blood screening kits respectively.

2.7.1 Sample Analysis/Methodologies

OnSite *H. pylori* Ag Rapid Test (USA), ROSTEC Rapid Diagnostic Test, for Fecal Occult Blood (FOB), Pasteur pipette, test card, universal sterile container and forceps.

2.7.2 Principle

The *H. Pylori* Antigen test is a qualitative membrane based immunoassay for the detection of *H. pylori* antigen in blood or stool sample. In this test procedure, *H. Pylori* antigen is immobilized in the test line region of the test. After specimen is added to the specimen well of the device, it reacts with specific *H. Pylori* antigen in the patient's blood or stool sample. This mixture migrates chromatographically along the length of the test and interacts with the immobilized antigen. If the specimen contains *H. Pylori* antigen, a colored line will appear in the test line region indicating a positive result. If the specimen does not contain *H. Pylori* antigen, a colored line will not appear in this region indicating a negative result. To serve as a procedural control, a colored line will always appear in the control line region, indicating that proper volume of specimen has been added and membrane wicking has occurred.

2.7.3 Procedure

1. The pouch was brought to room temperature before opening it. The test device was then removed from the sealed pouch and used as soon as possible.

2. The test device was placed on a clean and level surface. A drop of stool sample already emulsified in a buffered solution was added to the specimen pad of the test device and allowed to stand for 15minutes.
3. The results were subsequently read after 15 minutes

2.8 Statistical Analysis

Data generated from this study were statistically analyzed using MS Excel 2007 for defining the percentage frequency of *Helicobacter pylori* amongst patients presenting with presumptive gastritis in Rivers State University Teaching Hospital Port Harcourt. Data were represented in tables.

3.RESULTS

A total of 242 male and female patients attending the GIT clinic of Rivers State University Teaching Hospital (RSUTH) were recruited for this study. One Hundred (100) of the participants representing 41.3% were male while 142 participants representing 58.7% were female. Thirty(30) of the participants representing 12.4% were less than 18 years while those between the ages of 19-28, 29-38, 39-48, 49-60 and greater than 60 years and they were 30 (12.4%), 40 (16.5%), 54 (22.3%), 45 (18.6%), 36 (14.9%) and 37 (15.3%) respectively. Details are shown in table 1 below.

Table 1: Demographic Details of Participants

Parameters	Frequency	Percentage (%)
Sex		
Males	100	41.3
Females	142	58.7
Age Ranges		
≤18	30	12.4
19-28	40	16.5
29-38	54	22.3
39-48	45	18.6
49-60	36	14.9
≥ 61	37	15.3

Distribution of *H. Pylori* among Studied Participants as presented on figure 1 showed that 132 of the total participants representing a prevalence percentage rate of 55% were positive for *H. pylori* while 110 of the total participants representing 45% were negative.

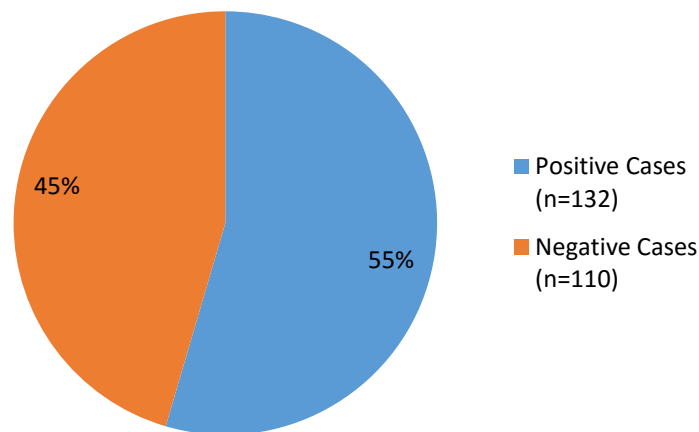


Figure 1: Distribution of *H. pylori* among Studied Participants

Distribution of *H. Pylori* among Studied Participants with respect to sex as presented on table 2 showed that 44 of the total male participants representing a percentage prevalence of 18.2% in male were positive for *H. Pylori* while 56 of the total participants representing 23.1% were negative for *H. Pylori*. Eighty eight (88) of the total female participants representing 36.4% were positive for *H. Pylori* while 54 representing 22.3% were negative for *H. Pylori*. Also distribution of *H. Pylori* among Studied Participants with respect to age showed that 19 of the total population below 18years were positive for *H. Pylori* representing a percentage prevalence of 7.9% while 11 of the participants below 18 years representing 4.5% were negative for *H. Pylori*. Those between the age group of 19-28, 29-38, 39-48, 49-60 and >60 years had a percentage prevalence of 9.5%, 10.3%, 10.7%, 8.3% and 7.9% positive cases respectively and 7.0%, 12.0%, 7.9%, 6.6% and 7.4% of negative cases respectively

Table 2: Distribution of *H. Pylori* among Patients with Respect to Demographic**Details**

Variable	Frequency	Percentage (%)
Males		
Positive Cases	44	18.2
Negative Cases	56	23.1
Females		
Positive Cases	88	36.4
Negative Cases	54	22.3
Age Ranges		
≤18		
Positive Cases	19	7.9
Negative Cases	11	4.5
19-28		
Positive Cases	23	9.5
Negative Cases	17	7.0
29-38		
Positive Cases	25	10.3
Negative Cases	29	12.0
39-48		
Positive Cases	26	10.7
Negative Cases	19	7.9
49-60		
Positive Cases	20	8.3
Negative Cases	16	6.6
≥ 61		
Positive Cases	19	7.9
Negative Cases	18	7.4

Distribution of FOB among Studied Participants as presented on figure 2 showed that 106 of the total participants representing of 44% of the total population were positive for FOB while 136 of the total participants representing 56% were negative.

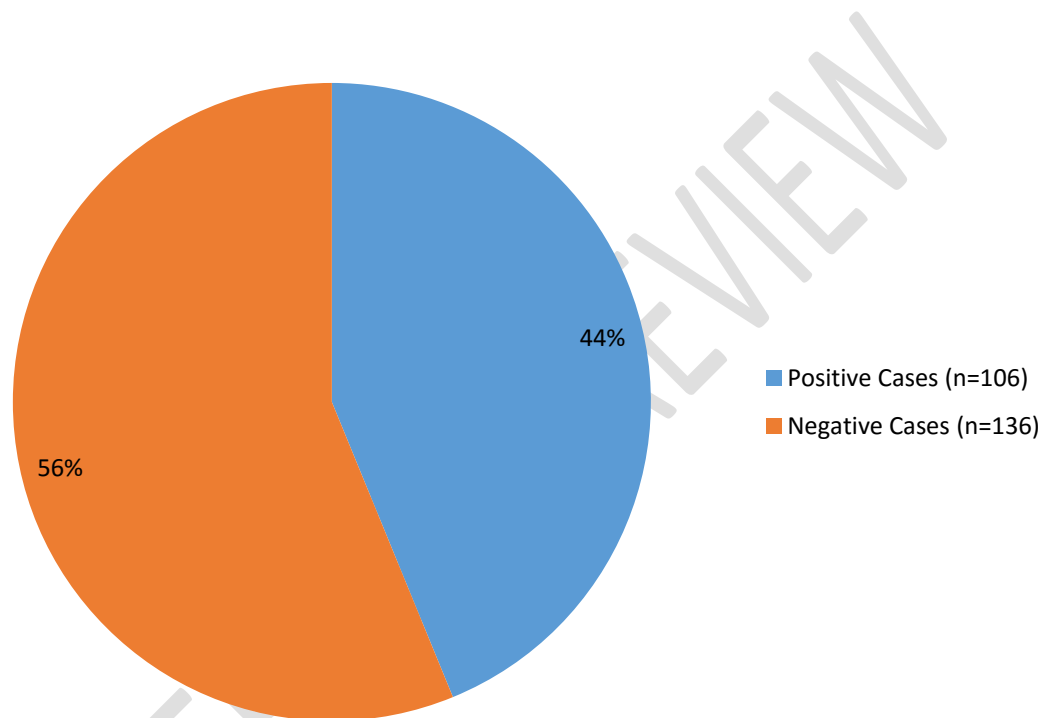


Figure 2: Distribution of Fecal Occult Blood (FOB) among Studied Participants

Relationship between gastritis symptoms and *H. Pylori* infection as presented on table 3 showed that 145 (59.9%) of the total participants had nausea of which 123 (50.8%) were positive and 22 (9.1%) negative for *H. Pylori* respectively. Participants without nausea symptoms were 97 (40.1%) of which 9 (3.7%) and 88 (36.4%) were positive and negative respectively. 116 (47.9%) of the total participants were vomiting of which 97 (40.1%) were positive and 29 (12.0%) negative for *H. Pylori* respectively. Participants without vomiting were 126 (52.1%) of which 32 (13.2%) and 84 (34.7%)

were positive and negative respectively. One hundred and ninety eight (198) which is 81.8% of the total participants were belching of which 129 (53.3%) were positive and 69 (28.5%) negative for *H. Pylori* respectively. Participants without belching symptoms were 44 (18.2%) of which 3 (1.2%) and 41 (16.9%) were positive and negative respectively. One hundred and fifty five (155) which is 64.1% of the total participants had abdominal pain of which 130 (53.7%) were positive and 25 (10.3%) negative for *H. Pylori* respectively. Participants without abdominal pain were 87 (36.0%) of which 2 (0.8%) and 85 (35.2%) were positive and negative respectively. One hundred and eighty five (185) which is 76.5% of the total participants had loss of appetite of which 119 (49.2%) were positive and 66 (27.2%) negative for *H. Pylori* respectively. Participants without loss of appetite were 57 (23.6%) of which 13 (5.4%) and 44 (18.2%) were positive and negative respectively. One hundred and seventy eight (178) which is 73.6% of the total participants had unexplained weight loss of which 100 (41.3%) were positive and 78 (32.3%) negative for *H. Pylori* respectively. Participants without unexplained weight loss were 64 (26.5%) of which 32 (13.2%) and 32 (13.2%) were positive and negative respectively. One hundred and nine (109) which is 45.0% of the total participants had stomach bleeding of which 107 (44.2%) were positive and 2 (0.8%) negative for *H. Pylori* respectively. Participants without stomach bleeding were 133 (55.0%) of which 25 (10.3%) and 108 (44.6%) were positive and negative respectively.

Table 3. Relationship between Gastritis Symptoms and *H. Pylori* Infection

Gastritis symptoms	Number Tested (%)	<i>H. Pylori</i> Cases	Frequency	Percentage (%)
Nausea				
Yes	145(59.9%)	Positive	123	50.8
		Negative	22	9.1
No	97(40.1%)	Positive	9	3.7
		Negative	88	36.4
Vomiting				
Yes	116(47.9%)	Positive	97	40.1
		Negative	29	12.0
No	126(52.1%)	Positive	32	13.2
		Negative	84	34.7
Belching				
Yes	198(81.8%)	Positive	129	53.3
		Negative	69	28.5
No	44(18.2%)	Positive	3	1.2
		Negative	41	16.9
Abdominal pain				
Yes	155(64.1%)	Positive	130	53.7
		Negative	25	10.3
No	87(36.0%)	Positive	2	0.8
		Negative	85	35.2
Loss of appetite				
Yes	185(76.5%)	Positive	119	49.2
		Negative	66	27.2
No	57(23.6%)	Positive	13	5.4
		Negative	44	18.2
Unexplained weight loss				

Yes	178(73.6%)	Positive	100	41.3
		Negative	78	32.3
No	64(26.5%)	Positive	32	13.2
		Negative	32	13.2
Stomach bleeding				
Yes	109(45.0%)	Positive	107	44.2
		Negative	2	0.8
No	133(55.0%)	Positive	25	10.3
		Negative	108	44.6

Relationship between *H. Pylori* Antigen and FOB as presented on table 4 showed that 65 of the total participants representing 26.9% were positive for both *H. Pylori* antigen and FOB while 69 of the total participants representing 28.5% of the total participants were negative for both *H. Pylori* and FOB. 67 of the total participants representing 27.7% of the total participants were positive for *H. Pylori* and negative for FOB while 41 of the total participants representing 16.9% were negative for *H. Pylori* antigen and FOB.

Table 4. Relationship between *H. Pylori* Antigen and FOB

Variable	Frequency	Percentage (%)
Positive Case for both <i>H. Pylori</i> Antigen and FOB	65	26.9
Negative Case for both <i>H. Pylori</i> Antigen and FOB	69	28.5
Positive Case for <i>H. Pylori</i> Antigen and Negative FOB	67	27.7
Negative Case for <i>H. Pylori</i> Antigen and Positive FOB	41	16.9

Antigen and Positive FOB

4. DISCUSSION

A cross-sectional, hospital-based study aimed at determining the prevalence of *Helicobacter pylori* amongst patients presenting with presumptive gastritis was carried out at gastroenterology clinic of Rivers State University Teaching Hospital Port Harcourt. Two hundred and forty (242) male and female participants were recruited for the study, their stool samples were analyzed using *H. Pylori* antigen test and fecal occult blood test kits, while socio demographic characteristics and symptoms for gastritis was assessed with pretested questionnaires. The prevalence of *H. Pylori* was found to be 55% in Rivers State University teaching Hospital (RSUTH). The result of this study is similar to 54% reported by Ishaleku and Ihiabe [32] who sampled 200 students in Nasarawa State, Nigeria. It is also comparable with the 50% projected world population prevalence for *H. pylori* infection [33] and also with 52% prevalence reported by Nwachukwu *et al.*, [2] at Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria. The prevalence reported in this study is lower than 81% reported by Bashir and Ali,[21] in Kano (Nigeria), 87% reported by Malu *et al.*, [34] in Jos (Nigeria), 73% reported by Aboderin *et al.*, [24] in South-West Nigeria, 77% reported by Mustapha *et al.*, [35] in Gombe and 73% reported by Ndububa *et al.* [36] in Ile-Ife. Also, the prevalence in this study is higher than 19.6% reported by Ayodele *et al.*, [23] at University of Port Harcourt Teaching Hospital. It is also higher than 1.2% to 12.2% variation reported in developing countries [19].

Various risk factors like living in crowded conditions, living without a reliable supply of clean water and living with someone who has an *H. pylori* infection [2, 37] which are the common lifestyles of study participants may be responsible for the increased infection and it remain a major health concern. However, awareness of the risk factors pertaining to *H. pylori* and lifestyles of the residents of Rivers State may contribute to the low prevalence reported in this study as compared to studies reported in other states. Also, variation in the infection prevalence of *H. pylori* as reported in our study and other studies may be as a result of the difference in method adopted for the diagnosis of the infection, prevailing public health indices of the sampled population and recruitment criteria as was also observed by Magalhães-Queiroz and Luzzo [33] and Hoang *et al.*, [38] in some developing countries. In this study, the prevalence of *H. Pylori* infection is higher in females (36.4%) than the males (18.2%) as seen in table 4. This result is in agreement with results obtained by Nwachukwu *et al.*, [2] who reported a higher prevalence in females than male. Similar report was also obtained from a study in Warri, Nigeria, which reported a higher *H. pylori* prevalence in females than in males [39]. Odete *et al.*, [40] also reported that the prevalence rate of *H. pylori* was higher in female than in the male. In contrast, Omosor *et al.*, [41] revealed the prevalence of *H. pylori* infection to be higher in males (55%) than females (51.4%). Woodward *et al.*, [42] also reported a higher prevalence of *H. pylori* in men than in women. Ford and Axon [26] observed that male gender is a risk factor for *H. pylori* infection. But Mutaz *et al.*, [43] in their study reported that *H. Pylori* Infection rates are similar in males and females.

The prevalence of the infection varies according to different ages, socioeconomic strata and geographical regions [25, 26]. Nwachukwu *et al.*, [2] reported a prevalence of 52% with the infection higher in female (53%) than the male (47%) with no

significant difference. Among the children, prevalence was 22%, the adults 60% and the elderly 35%. In this study, prevalence of *H. Pylori* was 7.9% in individuals below 18years, while those between the age group of 19-28, 29-38, 39-48, 49-60 and >60 years had a percentage prevalence of 9.5%, 10.3%, 10.7%, 8.3% and 7.9% respectively. These results showed that there is an age dependent increase in the rate of infection in individuals between the age group of 0-48years with a decline in infection rate as the individual approaches 49years of age.

An age-related increase of the prevalence of *H. pylori*, irrespective of the economic state of the country, was observed by several independent studies across the world [44, 45, 46]. A study carried out in Nnewi also considered age in relation to *H. pylori*, and discovered a prevalence rate of 22% in the children [2]. In developed countries, less than 10% of children younger than 12 years are infected but increases with age at a rate of 0.3% to 1% per year.

The incidence is 3% to 10% of the population each year in developing countries compared with 0.5% in developed countries [47]. Children differ from adults with respect to *H. pylori* infection in terms of the prevalence of the infection, *H. pylori* is the most important cause of peptic ulcer in adult population [48].

Studies of seropositivity in adults in developed countries revealed prevalence of 30% to 50%. This study reported increased prevalence in adults, which is sequel to the report that the prevalence of *H. pylori* is high in developing countries; reasons may be due to social economic difference and lifestyle. Elderly patients suffer from more serious complications resulting in higher hospitalization and mortality rates [49]. Studies conducted in the past decade have reported a high prevalence of *H. pylori* infection within the oldest population, especially in institutionalized old people, with a prevalence ranging from 70% to 85% [50, 51]. A marked reduction in the

prevalence (8.3 and 7.9%) of infection was noted in elderly people in this study. Although *H. pylori* infection is important in gastrointestinal diseases affecting all age groups, only a few studies have been published regarding elderly people [51]. Epidemiologic studies report higher prevalence of *H. pylori* infection in elderly with a ratio of over 70% in patients with gastrointestinal diseases and approximately 60% in asymptomatic patients [51, 52].

Relationship between gastritis symptoms and *H. Pylori* infection as presented on table 4. showed 50.8% prevalence of *H. Pylori* in those with nausea symptoms, 40.1%, 53.3%, 53.7%, 49.2%, 41.3% and 44.2% in those with symptoms of vomiting, belching, abdominal pain, loss of appetite, unexplained weight loss and stomach bleeding respectively showing a positive relationship between these symptoms and *H. Pylori* infection, although some individuals might still be having these symptoms and yet not positive for *H. Pylori* or not having gastritis. This may be as a result of other underlying conditions which share similar symptoms with gastritis. In other settings, gastrointestinal symptoms in patients such as; vomiting, heartburn, postprandial fullness and early satiety had no influence on infection status [53, 54, 55]. However, heartburn and belching have been found associated with seropositivity in Sudan [56] and Iran [57] respectively.

Conclusion.

This study's prevalence of *H. Pylori* was found to be 55% in Rivers State University teaching Hospital (RSUTH) with a higher prevalence among females than males and a age dependent increase in prevalence but decline above 49years. Therefore, it is imperative for proactive action to be taken in our homes, community and societies at large on public hygiene, food hygiene as well as public health and community sanitation. Proper education of citizens on the modes of transmission and prevention

of *H. pylori* infection is required to halt the trend of infection of this bacteria in our society.

Also, as complications of *H. pylori* infection cannot be over emphasized and increases with age [58]. In other words, this infection now has a higher incidence in the later stages of childhood, adolescence, adulthood and elderly [59]. Nevertheless, *H. pylori* testing should be regarded as an important aspect in clinical practice to help in treatment and better eradication of the infection in patients; this will lead to a significant decrease in gastritis in patients of different age group whether with symptoms or asymptomatic.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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