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Socio-demographic Factors and the rate of *Helicobacter pylori* infection Using Different Diagnostic Techniques among Patients in Rivers State, Port Harcourt, Nigeria

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Abstract

Background: In 2015, an estimated 50% of the world's population had *Helicobacter pylori* in their upper gastrointestinal tracts. Although *Helicobacter pylori* infection usually shows no symptoms, it has been regularly implicated as a cause of gastritis or stomach ulcers on the first part of the small intestine. This study aims to determine the socio-demographics relationship wi*th Helicobacter pylori* infection rate using different diagnostic techniques. **Methodology:** A total of 180 subjects between the ages of 1 and 60 years, who visited 2 teaching hospitals and 1 private hospital in Port Harcourt Metropolis for medical treatment were recruited. Convenience sampling techniques were used, and subjects' demographic information was obtained using a questionnaire. Ethical approval to use human subjects for this study was gotten and informed consent obtained from the study participants. Samples collected were blood and stool specimens. **Results:** This study established the presence of *H. pylori* infection among subjects in some locations in the Port Harcourt metropolis with a prevalence rate of 44% out of 180 blood samples tested for *H. pylori* by serology, 8.3% tested positive using stool antigen and 5.2% tested positive with PCR technique respectively. Females had prevalence rates of 53%, 11%, and 4.0%, by serology, stool antigen, and PCR methods respectively, while males had prevalence rates of 33%, 5.0%, and 1.2% respectively, showing a higher prevalence rate in females than males. Also, adult participants aged between 16 and 30 years had high prevalence of *H. pylori* at 52.9% while, those within the age range of 30 and above 63 years had a prevalence of 46%. **Conclusion:** The result from this research affirms the presence of *Helicobacter pylori* in subjects within Port-Harcourt, with a lower prevalence rate of the infection seen in males than females.

Keywords: *Helicobacter pylori, diagnostic technique, serology,, PCR*

Introduction

Helicobacter pylori is a spiral-shaped bacterium that infects roughly half the world's population. *Helicobacter pylori* was discovered in the stomach of a patient with gastritis and ulcers in the year 1982 by two doctors Barry Marshall and Robin Warren of Perth in Western Australia [1]. This discovery helped the researchers to link the *Helicobacter pylori* infection to conditions like gastritis, ulcers, and stomach cancer. Before the time of discovery, there was a conventional thinking that no bacterium could live in an acidic environment of the human stomach, since the human stomach is an unfriendly place for most infective bacteria due to the very low pH in the place. The Australian's discovery significantly changed the concept of gastric microbiology.

Helicobacter pylori infection usually has no symptoms, but most times causes gastritis which is an inflammation of the lining of the stomach, 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 [2]. *Helicobacter pylori* has been noticed to cause a wide range of other diseases [3] It also plays an important role in the natural stomach ecology, e.g. by influencing the type of bacteria that colonize the gastrointestinal tract [2].

In 2015, it was estimated that over 50% of the world's population had *Helicobacter pylori* in their upper gastrointestinal tracts [4]. The colonization of this infection is more common in developing countries [5]. In recent decades, *Helicobacter pylori* colonization of the gastrointestinal tract has declined in many countries [6].

The world population is infected by the bacterium, and the most widespread infection in the world [7]. There is a wide variation in the prevalence of *H. pylori* between regions and countries. The highest prevalence rate is in Africa (79.1%), Latin America and the Caribbean (63.4%), and Asia (54.7%) [8]. The lowest *H. pylori* prevalence in Northern America was (37.1%) and oceanic (24.4%) [78].

In the Eastern part of Africa, a study from Kenya, patients who were presented with dyspepsia, showed a prevalence of *H. pylori* infection of 73.3% in children and 54.8% in adults. The authors reported *H. pylori* prevalence of 53% in the dyspeptic patients in Addis Ababa with an estimated prevalence rate in patients aged between 54-61 years [9].

In Nigeria, various studies on *H. pylori have* shown prevalence rates between 73.0% and 94.5% among patients with dyspepsia [10].

The age at which an individual acquires this bacterium influences the pathologic outcome of the infection. People who are infected at an early age are likely to develop more intense inflammation that may be followed by atrophic gastritis, with a higher subsequent risk of gastric ulcer, gastric cancer, or both [11]. Acquisition at an older age brings different gastric changes which are more likely to lead to duodenal ulcers [10]. The *H. pylori* infection is usually acquired in early childhood in all countries [12] the infection rate of children in the developing nations is higher than as compared to the industrialized nations which is probably due to poor sanitary conditions.

There is a higher prevalence among the elderly which reflects higher infection rates in the past when the individuals are children rather than the more recent infection at a later age of the individual [12]. In the United States, the prevalence rate appears higher in African American and Hispanic populations which is most likely due to socioeconomic factors [13]. The widening differential gap in prevalence has an important implication for the future worldwide prevalence of sequelae associated with *H. pylori*, which includes peptic ulcer disease and gastric cancer [13]. The differences in *H. pylori* prevalence are likely to reflect in urbanization, sanitation access to clean water, and varied socioeconomic status [13].

Helicobacter pylori infection maintains a fast-growing prevalence and is of public health concern. Thus, there is a need for the evaluation of available diagnostic techniques

that can validate or undermine the efficiency of diagnosis in a resource-poor setting.

This study aims at detecting *Helicobacter pylori* using different diagnostics techniques. The information obtained from the study will inform Health Policymakers, Public Health enthusiasts, Health Workers, and Researchers alike on the prevalence of, and the best ways of monitoring and diagnosing *Helicobacter pylori* infection.

MATERIALS AND METHODS

Study Area

The study was carried out in Rivers State University Teaching Hospital, Port Harcourt, Nigeria which is located between Latitude $4^0$46,49˝N and $7^0$0'50" E Longitude $4^0$780 28^0N and 7.01389^{0}E, the University Teaching Hospital Port Harcourt which is located between Latitude $4^0$53/58' and $6^0$55'43" and Longitude 4.89944^{0}N and 6.92861^{0}E and Rehoboth Specialist Hospital which is in D/line, Port Harcourt Metropolis. Analysis of samples was conducted at the Rivers State University Medical Centre Laboratory in Nkpolu-Oroworokwo, Port Harcourt, a branch of the Rivers State University Teaching Hospital in Port Harcourt. Its departments include Pharmacy, Finance, Emergency unit, Physiotherapy unit, Radiology, Maintenance and General Administration.

Study Design

The study is a cross-sectional study involving patients who visited the hospital for healthcare purposes. A total of one hundred and eighty (180) subjects between 1 to 60 years and above were recruited for the study. The subjects visited the University of Port Harcourt Teaching Hospital, Rivers State University Teaching Hospital, and Rehoboth Specialist Hospital in Port Harcourt Metropolis for medical treatment. Samples collected were blood (180) and stool (180), making a total of 360 specimens.

Ethical Consideration

Ethical approval "RSUTH/REC/2022189" dated 24/08/2022 was obtained from Rivers State University Teaching Hospital Ethics Committee after the study proposal was considered. Also, written consent was obtained from the subjects indicating willingness to participate in the study.

Eligibility Criteria

The study included healthy subjects with no evidence of infection nor other chronic diseases, subjects who attended the hospital with abdominal pain and heartburn, and symptomatic or asymptomatic subjects ranging from 1 to 60 years and above.

Those excluded are **s**ubjects who didn't attend the hospital, or immune-compromised individuals with evidence of chronic infection like cancer, HIV, and diabetes, and individuals who did not give their consent.

Sampling Method

The convenience sampling technique was explored. Demographic information of subjects was obtained using a well-structured questionnaire. The demographic information obtained includes age, place of residence, educational status, antibiotics medication, antiretroviral medication.

Sample collection

Blood and stool samples were collected from consenting subjects. Three hundred and sixty (360) samples were collected of which 180 were faecal specimens and 180 were blood specimens. Five (5 mL) venous blood sample was collected into plain bottle from each participant and the serum was separated immediately into sterile tubes and stored at a temperature of 2˚C - 8˚C for up to 2 days before analysis, until analyzed for anti-Helicobacter *pylori* antibodies—IgG detection. Also, Stool specimens were collected into sterile plain wax-free crew-capped container from the participants. Small piece of stool (~5 mm in diameter; ~150 mg) on different days of visits for the outpatients. All samples were analyzed

in the laboratory after which molecular analysis was carried out.

Culture Method

The media used for this research work included Columbia agar and Mueller Hinton agar were used for isolation. The faecal samples were examined physically, and the colour and consistency, whether formed, semi-formed, or watery, presence of blood/mucus was all noted and reported. Each specimen was inoculated onto Columbia Agar (Mast Diagnostica GnbH, Feldstrasse 20, DE 23858, Reinfeld, Germany) which is enriched with sheep blood, they were also inoculated on Mueller Hinton agar enriched with sheep blood. After streaking onto both media plates, they were incubated under microaerophilic conditions at 37℃ for 2 to 7 days.

Aseptic techniques were observed in all the steps of specimen collection and inoculation onto culture media to minimize contamination.

Pure cultures were obtained from the mixed population of the respective growth and were sub-cultured onto different fresh media using their specific media and the plates were incubated for another 24 to 48 hours at 35℃ to 37℃.

Identification of isolates was strictly based on the following: color, surface, texture, size, elevation margin, Chemical and biochemical analysis.

Gram staining and fixing of isolates were done after culture growth. The slides were air-dried and examined microscopically using an oil immersion objection lens at x100 magnification for gram-negative rod-shaped bacteria. (Cheesbrough, 2004)

Serology (Antibody Rapid Test kit), Antigen Rapid Test

A serology (serum antibodyIgG) test for *Helicobacter pylori* was carried out on the blood specimens while stool antigen for *Helicobacter pylori*, and molecular identification of the *H. pylori* positive stool specimens.

Statistical Analysis

The data from the study were imputed in Microsoft Excel sheet and exported to SPSS version 25.0 for descriptive and Chi-square analysis. The test was considered significant at a p-value less than 0.05

RESULTS

Table 1 shows the prevalence of *H. pylori* by gender using three techniques. The result shows that there was a significant difference (p-value<0.0003) by gender and *H. pylori* infection based on serological examination. Also, the result shows that there was a significant difference (p-value<0.035) by gender and *H. pylori* infection based on antigenic examination. Finally, there was a significant difference by gender and *H. pylori* infection based on PCR technique.

Table 1: Gender and Age Distribution of *Helicobacter pylori* based on Assay Method used in this study

Gender	Age (yrs)	Number Estimated	Serology number Positive n(%)	Antigen number Positive n(%)	PCR
Male	33±15	81	27(33.3)	5(6.2)	1(1.2%)
Female	35±11	99	53(5.1)	11(11.1)	4(4.0%)
Total		180	79(43.8)	15(8.3)	5(2.8%)
p-value			0.0003	0.0350	<0.001
Chi-square			15.43	6.53	10.611

Legend: PCR = Polymerase Chain Reaction

Table 2 shows the distribution of of *H*. pylori infection rate by various educational levels based on serological, antigenic and molecular techniques. Serological technique presented the highest rate in primary, secondary and tertiary while PCR technique had the least prevalence at all levels of educational levels.

Table 2: Distribution of *H. pylori* infection among educational levels

Variable	Serology n (%)	Stool Antigen n (%)	PCR n (%)
Education			
Primary (n=20)	14(70)	2(10)	1(5)
Secondary (n=88)	36(41)	3(3)	2(2.3)
Tertiary (n=72)	29(40)	10(19)	2(2.8)
Total = 180	79 (43.9)	15 (8.3)	5 (2.8)

Table 3 shows the relationship between prevalence of *H. pylor*i infection and age groups by three different analytical techniques. The result showed that there was a significant association (p-value <0.05) between *H. pylori* infection rate and age groups by the three different laboratory techniques used.

Table 3: Comparative Prevalence of *H. pylori* based on Age using Serology, Antigen and PCR

Age (years)	Total Number Examined	Serology Number positive %	Antigen Number positive positive %	PCR Number
1 – 10	5	0 (0)	0 (0)	0 (0)
11 – 20	26	9 (36)	2 (8)	1(3.8)
21 – 30	41	22 (54)	2 (5)	0 (0)
31 – 40	65	31 (48)	8(12)	2(3.1)
41 – 50	28	9 (32)	1 (4)	1(4)
51 – 60	11	6 (55)	0 (0)	0 (0)
≥ 61	4	2 (50)	2 (50)	1(25)
P-value		<0.0001	0.0002	<0.0001
Chi-square		26.67	26.75	2.6

Table 4 showed the distribution of *H pylori* by various study locations using different laboratory techniques. Based on laboratory techniques, Rehoboth had the highest rate of *H pylori* infection in serological assay, UPTH had the highest rate of *H pylori* infection in stool antigen test and PCR.

Table 4: Distribution of *H. pylori infection* across the studied Locations

Location	Total sample	Serology n (%)	Stool Antigen n (%)	PCR n (%)
UPTH	84	46 (55)	9 (11)	3(3.6)
RSUTH	66	12 (18)	3 (5)	1(1.5)
Rehoboth	30	21 (70)	3 (10)	1(3.3)
Total	**180**	**79 (44)**	**15 (8.3)**	**5(2.8)**

Key: UPTH – University of Port Harcourt Teaching Hospital, RSUTH – Rivers State University Teaching Hospital

DISCUSSION

Helicobacter pylori is one of the leading bacteria of interest in the stomach of approximately half of the world's population and it's capable of causing several gastrointestinal problems, including ulcers and much less commonly, stomach cancer. Several investigations done on *H. pylori* have suggested that poor hygiene, overcrowding and other risk factors such as non-filtered water, smoking facilitate the transmission of *H. pylori* infection. This study established the presence of *H. pylori* infection among subjects in some location in Port Harcourt metropolis with a prevalence rate of 44% from 180 blood samples tested for *H. pylori* by serology, 8.3% tested positive for stool antigen and 5.2% tested positive for PCR respectively.

However, this was low compared to prevalence rates obtained in studies conducted in other States in Nigeria. One such was a study conducted in Kano North-West, Nigeria, that had a prevalence rate of (96%) was reported by [14]. This could be because of deficient surveillance or lack of diagnostic materials in carrying out the test. Another study carried out in Delta State in South-South Nigeria by Omosor *et al*., [15] using serology and stool antigen methods recorded a high prevalence rate of 52%, and another study conducted Anambra State, South- East Nigeria by Chukwuma *et al*., [16] reported a prevalence rate of 51%. The findings from these studies may have had other factors influencing it like low standard of living, poor personal hygiene, overcrowding, level of education, lifestyle, and level of poverty Ajayi *et al*. [17]. In this study, females had higher prevalence rate of *H. pylori* infection using different assay methods used than male. Female had prevalence rate of 53%, 11%, and 4.0%, for serology, stool antigen and PCR methods, while males had prevalence rate of 33%, 5.0% and 1.2% respectively. This might be due to recruiting more females than males for the study, which agrees with the research done in Yemen by Almorish *et al.,* [18] but there was no significant difference by gender with P- value of 0.0003, 0.0350 and 0.0001 respectively. This research also agrees with study of Abdulrahman *et al.,* [19] who reported that in his study, females had higher prevalence of 76.4% and 50% in both blood antibodies and stool antigen method, while males had 68.7% and 43.7% respectively. Also, in a prevalence study conducted in Port Harcourt by Agi *et al.* [20] they reported high prevalence in female than male. Another study by Ibrahim *et al*. [21] and Ansari *et al*. [22] reported that male had higher rate of *H. pylori* infection than female and there was no significant relationship between sex and *H. pylori* infection.

Several studies have reported on the prevalence of *H. pylori* infection in Nigeria and other developing countries over time with difference in demography, age groups, occupation, gender, level of education, marital status all over world. But much of the prevalence rate of *H. pylori* infection has been carried out based on demography and religion as a risk factor.

In this study, different educational levels were shown to be at risk for *H. pylori* infection. The prevalence of *H. pylori* infection was higher among those who had primary educational level of 85%, compared to a study by Nguyen *et al*., [24] done in the North of Vietnam which indicated low parental education as a risk factor of acquiring *H. pylori*. This might be because of low awareness and exposure to the infection. Similar findings were also reported from other developing countries like Pakistan, Iran and China [25], pointing that patients who had low education level were at a higher risk of *H. pylori* infection. On the other hand, those in the educational lower classes were also associated with poor sanitation, overcrowding and lifestyle which was seen as a risk factor to *H. pylori* infection.

This study also showed that different age groups had different prevalence rates. The prevalence of 48.0% within the age group 1-20 years was higher than the ones within the same age group in Tanzania and Ghana in the age range of 6 months to 17years who had 11.5% and 14.2% prevalence rate of *H. pylori*

infection.[25]. Similar research was carried out by Etukudo *et al.* [26] with findings of 30.9% seropositive *H. pylori* infection among children within the age group of 0.5 to 15 years. Also, a study conducted a study in Lagos, South-west Nigeria reported a high seroprevalence of *H. pylori* 68.7% infection in children within the age group, which was higher than the one in our study, this could be due to infection associated with personal hygiene, household overcrowding, source of drinking water and type of toilet used [27]. In this study, the age distribution tends to increase the infection rate of *H. pylori* infection among the younger age group. Those aged 11-20 years showed a prevalence rate of 36% for serology, 8% for stool antigen, and 3.8% for PCR. The middle-aged group of 31-40 and 41-50 had a prevalence rate of 48%, 12%, 3.1%, 32%, 4%, and 4% for serology, stool antigen, and PCR respectively. The aged group 51 and above had 50%, 50%, and 25% respectively for the different test methods, however, there was no statistically significant difference in *H. pylori* prevalence among age groups with a p-value of < 0.0001, 0.0002, and 0.0001 used in the different assay method serology, Antigen and PCR respectively. In this research, adult participants aged between 16 and 30 years had the highest prevalence of *H. pylori* 37% and 52.9% respectively with participants within the age group 30 years and above 63%, 46%. This agrees with the present study result as adults less than 40 years showed the highest prevalence rate, this may be because of mobility and activity as well as lifestyle of this age group as they tend to be more sociable, mobile, and use fast food. However, the work done by Ayodele *et al.* [28] on age distribution of *H. pylori* infection with peak values of 6.88% in the 51-60 years age group did not conform with the published data of Ishaleku & Ihiabe [29] which showed a peak infection rate of 85.7% among adults aged 31-40 years dropped to 66.7% among those 41-50 years, but it was 28.6% in 51-years and above. The result, however, is in line with the published work of Abdulrahman *et al.,* [19] which had the highest positive result among the age group of 41-80yr (80%) blood antibodies and (60%) used and stool antigen test respectively, but the work of Ishaleku and Ihiabe [29] varied with the results of this research. However, the variation in the prevalence of *H. pylori* as reported in both cases may be because of differences in methods adopted for the diagnosis, prevailing public health indices of the sampled population, and recruitment criteria as was also observed by Queiroz *et al.* [30] and Hooi *et al*. [8] in some developing countries. Nevertheless, according to the World Organization of Gastroenterology [31], the prevalence of *H. pylori* varies between 85% and 95% in developing countries like Nigeria and 30% to 50% in developed countries. These Predictions agreed with the results obtained in this study for several reasons, one of which is the improvement in sanitation and the awareness of infection in recent times. The high prevalence of *H. pylori* infection found in ages from 30-60 years could be due to lifestyle of the organism. The people within this age range are active workers with little or no time to cook at home and this is traceable to consumption of contaminated food and unhygienic food handlers. Secondly, people within the same age range are sexually active, there is evidence of oral-oral transmission of *H. pylori.* [32].

Conclusion

Helicobacter pylori is a ubiquitous bacterium usually found in about 50% of the world's population. The result from this research affirms the presence of *Helicobacter pylori* in subjects within Port-Harcourt, with a lower prevalence rate of the infection seen in males than females.

Recommendation

It is recommended that Government and public health organizations intensify policy formulation, its implementation, and the enlightenment of citizens on the public health implications of high prevalence of *H. pylori* in rural and sub-urban communities.

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