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DETECTION OF *HELICOBACTER PYLORI* IN BLOOD AND STOOL SAMPLES: A COMPARATIVE STUDY OF ANTIBODY-BASED TESTS

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ABSTRACT

Helicobacter pylori infection is a significant global health challenge. Accurate diagnosis is crucial for effective management and treatment. Currently, various diagnostic methods are available, including antibody-based tests on blood and stool samples. However, the reliability and efficacy of these tests remain uncertain. This study focused on the detection of *H. pylori* in blood and stool samples using antibody-based tests. A total of 200 stool and blood samples were collected and screened for HP using an antibody-based assay. The study revealed that the Blood antibody test (48.90) significantly ($p \leq 0.05$) detected H. pylori more than the stool antibody test (19.40%), and the detection was mostly seen among female patients, patients of age 45 years and above, traders and those patients with no formal education. There was a significant ($p \leq 0.05$) increase in the detection of *H. pylori* among those patients who had stomach ulcers, heartburn, chest pain, epigastric pain, abdominal pain, inflammation and constipation, compared to healthy individuals. Therefore, the study has shown that *H. pylori* was significantly detected in blood

samples than in stool samples using antibody-based tests, and sociodemographic factors and medical history of the patients influenced the test

Key words: *Helicobacter*, *pylori*, Stool, Blood, Ulcer

INTRODUCTION

The quest to attaining a state where the rate of infectious diseases is drastically reduced has been a common goal of several pharmaceutical and medical microbiology researchers.

Helicobacter pylori is a Gram-negative, micro-aerophilic and spiral-shaped bacterium that belongs to the family *Helicobacteraceae*. The genus has several species, of which some are found in the upper gastrointestinal tract and liver of some mammals and birds. The species of the genus are motile due to the presence of flagellum (Savoldi *et al*., 2018).

Research has revealed that *H. pylori* is a highly pathogenic species of the genus, which infects mostly mammals especially man (Goderska *et al*., 2018). The infection that occurs due to the presence of the bacterium in the gastrointestinal tract has been recognized globally as a threat because the high level of disorderliness of the system is experienced by the infected individuals. The most debilitating aspect of the infection is that all age groups, occupations, and genders are vulnerable (Garrido-Trevino *et al*., 2022).

Several researchers have reported that the ability of the bacterium to cause severe infection with acute clinical manifestations could be attributed to the presence of virulent factors such as adhesins, which enable the organism to attach firmly to the mucosa of the stomach and urease, which enables it to breakdown urea, releasing ammonia and carbon dioxide (Ayala *et al*., 2014; Azadi *et al*., 2019).

Some researchers have purported that the ability of the pathogen to produce ammonia from urea provides a conducive environment for proliferation (Spinu *et al*., 2016; Mintah *et al*., 2019). The attachment of the organism in the mucosa enables it to destroy the epithelial cells in the tissue, thereby leading to bleeding in severe cases. Some of the infected patients had excreted the organism in faeces, which also provides relevant diagnostic information (Bouhenni *et al*., 2019). The wound caused by the organism is capable of depriving an infected person of several foods, especially when prepared using pepper, as it aggravates pain.

Several efforts had been made by health practitioners, especially those in pharmaceutical industries to ensuring that an ideal conventional antimicrobial agent is produced, which would curtail the menace (Ngan *et al*., 2021). It is

appalling that for decades, most of the drugs and antibacterial agents had not yielded the desired goal. To an extent, it was speculated that stomach ulcer caused by *H. pylori* has no cure but can only be checked. As a result of this, it has been difficult to have access to a drug that can totally cure ulcer infection, especially in developing countries, where the prevalent rate remains almost constant (Alibi *et al*., 2020). Therefore, this study was undertaken to compare the diagnostic performance of antibody-based tests in blood and stool samples for the detection of *H. pylori*.

MATERIALS AND METHODS

Study area: Nnewi North Local Government metropolitan commercial city and Uli in Ihiala local government Area both in Anambra State, Nigeria are towns of historic importance. Nnewi is located in the South-East zone in Anambra state, Nigeria. It is the second largest commercial city in Anambra State in South-Eastern Nigeria with two local government areas, Nnewi North and Nnewi South. Nnewi North is commonly referred to as Nnewi Central and is the centre of commercial activities. The city spans over 1,076.9 square miles (2,789 km^2) in Anambra State with four autonomous quarters: Otolo, Uruagu, Umudim, and Nnewichi. It has a land mass area of 60.0 $km^{2,}$ a density of 3, 428 /km^2 as of (2016). According to the World Bank, United Nations Census (2019), the population in urban areas as of 2016 was 1,004706, and 193,987 in city areas, though bearing in mind that all population figures for Nigeria show high error rates since census results are disputed. Nnewi metropolitan area and its satellite towns are home to nearly 2.5 million residents as of 2005 and Nnewi–North Local Government Area, with a land mass of 128 Km sq. an estimated number population of 121,063 and a population density of 946 (Anambra State Statistical Year Book, 2010). The average monthly maximum temperature was 27.46^{o}C and a minimum of 23.65 ^{0}C in 2010, and the annual monthly relative humidity was 79.66 by 2010. Nnewi is bounded in the west by Ekwusigo, northwest by Idemili, northeast by Aniocha, east by Aguata, and southwest by Ihiala local government areas. The occupation of the inhabitants includes trading. Uli is situated at the extreme southeast corner of Ihiala local government area of Anambra state in Nigeria.

Research design: This is cross-sectional research to find out the corollary of activities of some Nigerian medicinal

plant extracts against *Helicobacter Pylori* in Nnewi North Local Government Area and Uli in Ihiala Local Government Area. It is a hospital/school/Laboratory-based study.

Inclusion criteria: All willing subjects within the age range of 2 and above. Those not on antibiotics or herbal therapy one week before sample collection. Those attending clinics in the selected hospitals, Schools and laboratories in Nnewi metropolis and Uli town**:** Males and Females. Patients not on active immune suppressive therapy, coagulation drugs or showing coagulation effect, and malignant diseases (cancer) or are allergic to drugs used.

Exclusion criteria: Unwilling patients. Children below 2 years of age. Those on antibiotics or herbal therapy three weeks before sample collection. Those with a history of underlying diseases like diabetes, asthma, physical or mental impairment, pregnant or breastfeeding women. Patients on active immune suppressive therapy, proton pump inhibitors or Pepto-Bismol for at least 2 weeks, coagulation drugs or showing coagulation effect, and malignant diseases (cancer) or signs of allergy to drugs used.

Study population: The study consists of a total of two hundred and fifty (250) participants of the age range of 2 years and above.

Sampling technique: The purposive sampling technique was used to recruit participants. Those who presented the symptoms and signs that were suggestive of ulcers or gastritis. They were educated about the study and those who were willing to participate gave their consent in writing, and that of their parents/guardian until the required sample size was attained.

Data collection: A standard questionnaire consisting of 9 items written in understandable language and related to the *H. pylori* infection symptoms as noted by (Bisbal-Murrugarra *et al.,* 2002) was used. A structured questionnaire to collect information about the research subjects. It is an open-ended questionnaire which was anonymously answered voluntarily by the respondents before the commencement of the exercises. The questionnaire includes a system of qualification levels for each symptom taking into account the frequency and intensity of presentation in the previous two weeks. Three criteria were used to define, the questionnaire; data on personal, demographical variables,

environment and lifestyle factors were obtained using a structured questionnaire with closed and open-ended questions. These were answered under the guidance of the researcher at this point.

Sample collection: Clinical samples of blood and stool were used for the analysis. Before the collection oral consent was obtained from participants. Blood samples were collected by vein-puncture method from the anti-cubital fossa of the hand. Four milliliters (4 mL) of blood was drawn from each participant, dispensed into a non-anticoagulated container and allowed to clot. \Sterile plastic stool containers without preservatives were given to each subject and they were instructed to collect stool specimens following preclusive measures as described by (Cheesbrough, 2010; Ekesiobi *et al*., 2025a; Ekesiobi *et al*., 2025b; Ekesiobi *et al*., 2025c; Ekesiobi *et al*., 2025d). The collected samples were kept inside the cooler containing an ice pack, and the samples were transported to the laboratory for immediate analysis.

Screening for the Presence of *H. pylori* using Immunological Kits

Sample processing: Blood samples were allowed to clot and the clothed blood was retracted and centrifuged (Thermo Scientific, England) at 4000 revolutions per minute (r.p.m) for 10 minutes. Sera separated from cells after centrifugation was used for the *H. pylori* rapid test. (Sera can be stored in Eppendorf tubes at -20 ° C and analyses carried out within one week of collection). The stool samples were collected, covered immediately kept in a cooler containing ice block, and transported to the laboratory for immediate analysis.

***H. pylori* stool antigen test (HpSA):** *H. pylori* stool antigen test kit (SD$^{\text{BIOLINE}}$ *H. pylori* Ag, Germany) method was used. The assay was carried out according to the manufacturer's instructions. Three (3) mls of the assay diluent was transferred into the desired collection tube for use and about one gram of faecal sample was emulsified into it with the sample collection swab stick provided. The swab was swirled not less than 10 times. The collection swab was discarded after squeezing against the wall of the collection tube. The resulting suspension was allowed to stand for 5 minutes, and three drops of the suspended supernatant were added into the sample well(s) of the cassette test device and left to run for fifteen minutes. The appearance of two test lines that is: "C" and "T" is the

Control and the Test lines in the result window, indicating **positive results**; While one test line, "C" indicates a **negative result**.

Statistical Analysis

The results of the data generated were expressed in percentages, tables and figures. The significance of the prevalence and susceptibility study were determined using Analysis of variance (ANOVA) at a 95% confidence level. Pairwise comparison was carried out in an Excel sheet using the student "t" test (Iheukwumere *et al*., 2020; Ekesiobi *et al*. 2025e; Ekesiobi *et al*., 2025f; Ekesiobi *et al*., 2025g; Iheukwumere *et al*., 2025a; Iheukwumere *et al*., 2025b; Iheukwumere *et al*., 2025c; Iheukwumere *et al*., 2025d; and Iheukwumere *et al*., 2025e)

RESULTS

The study has shown age, sex, marital status, occupation, educational level and medical history influenced the detection of *H. pylori* in blood and stool samples as shown in **Tables 1-6**. Detection of *H. pylori* was detected most among the single female participants, who had no formal education, were traders, 45 years old and above, and had stomach ulcers. Also, the study showed that the detection of *H. pylori* using antibody-based kits was seen among the participants who had medical history, and these were statistically significant ($p \leq 0.05$).

Table 1: Detection of *H. pylori* based on Age

Age		BAT		SAT	
	N	P (%)	N (%)	P (%)	N (%)
14 years and below	70	20 (28.57)	50 (71.43)	8 (11.43)	62 (88.57)
15-24 years	51	26 (50.98)	25 (49.02)	7 (13.73)	44 (86.27)
25-34 years	27	15 (55.56)	12 (44.44)	4 (14.81)	23 (85.19)
35-44 years	23	16 (69.57)	7 (30.43)	6 (26.09)	17 (73.91)
45 years and above	29	21 (72.41)	8 (27.59)	14 (48.28)	15 (51.72)
Total	200	98 (49.00)	102 (51.00)	39 (19.50)	161 (80.50)

Table 2: Detection of *H. pylori* based on Sex

Sex		BAT		SAT	
	N	P (%)	N (%)	P (%)	N (%)
Male	89	41 (46.07)	48 (53.93)	11 (12.36)	78 (87.64)
Female	111	57 (51.35)	54 (48.65)	28 (25.23)	83 (74.77)
25-34 years	200	98 (49.00)	102 (51.00)	39 (19.50)	161 (80. 50)

Table 3: Detection of *H. pylori* based on marital status

Status		BAT		SAT	
	N	P (%)	N (%)	P (%)	N (%)
Single	97	58 (59.79)	39 (40.21)	22 (22.68)	75 (77.32)
Married	54	21 (38.89)	33 (61.11)	9 (16.67)	45 (83.33)
Widow/widower	31	13 (41.94)	18 (58.16)	6 (19.35)	25 (80.65)
Divorce/separated	18	6 (33.33)	12 (66.67)	2 (11.11)	16 (88.89)
Total	200	98 (49.00)	102 (51.00)	39 (19.50)	161 (80.50)

Table 4: Detection of *H. pyori* based on occupation

Occupation	BAT	SAT

	N	P (%)	N (%)	P (%)	N (%)
Student	77	37 (48.05)	40 (51.95)	11 (14.29)	66 (85.71)
Trader	39	27 (69.23)	12 (30.77)	18 (66.67)	21 (33.33)
Civil servant	27	9 (33.33)	18 (66.67)	3 (11.11)	24 (88.89)
Teacher	19	3 (15.79)	16 (84.21)	1 (5.26)	18 (94.74)
Health worker	22	3 (13.64)	19 (86.36)	1 (4.55)	21 (95.45)
Unemployed	16	9 (56.25)	7 (43.75)	5 (31.25)	11 (68.75)
Total	200	98 (49.00)	102 (51.00)	39 (19.50)	161 (80.50)

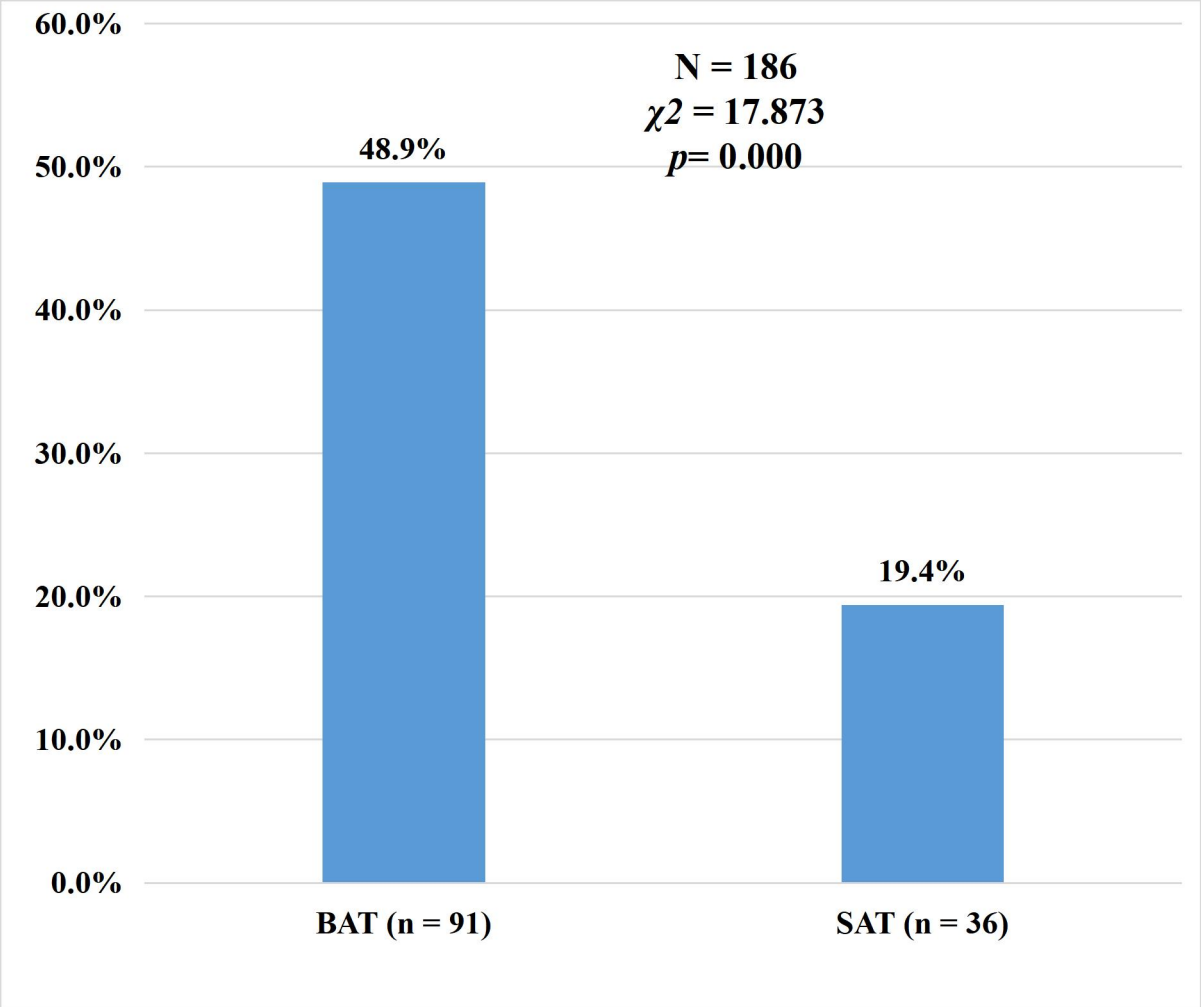
Table 5: Detection of *H. pylori* based on educational level

Educational Level		BAT		SAT	
	N	P (%)	N (%)	P (%)	N (%)
Primary	27	14 (51.85)	13 (48.15)	11 (40.74)	16 (59.26)
Secondary	42	20 (47.62)	22 (52.38)	8 (19.05)	34 (80.95)
Tertiary	105	47 (44.76)	58 (55.24)	6 (5.71)	99 (94.29)
No formal education	26	17 (65.38)	9 (34.62)	14 (53.85)	12 (46.15)
Total	200	98 (49.00)	102 (51.00)	39 (19.50)	161 (80.50)

Table 6: Detection of *H. pyori* based on medical history

Medical history		BAT		SAT	
	N	P (%)	N (%)	P (%)	N (%)
Chest pain	28	16 (57.14)	12 (42.86)	5 (17.86)	23 (82.14)
Heart burn	31	15 (48.39)	16 (51.61)	2 (6.45)	29 (93.55)
Epigastric pain	14	9 (64.29)	5 (35.71)	4 (28.57)	10 (71.43)
Stomach ulcer	43	34 (79.07)	9 (20.93)	21 (48.84)	22 (51.16)
Constipation	23	12 (52.17)	11 (47.83)	4 (17.39)	19 (82.61)
Inflammation	19	11 (57.89)	8 (42.11)	3 (15.79)	16 (84.21)
Normal	42	1 (2.38)	41 (97.62)	0 (0.00)	42 (100.00)
Total	200	98 (49.00)	102 (51.00)	39 (19.50)	161 (80.50)

The prevalence of *Helicobacter pylori* as determined via SAT and BAT is shown in **Figure1** below. BAT and SAT recorded a prevalence of 48.9% and 19.4%, respectively, which is statistically significantly difference (P<0.00).



DISCUSSION

The influence of age, sex, marital status, occupation, educational level and medical history of the participants in the detection of *H. pylori* corroborated the report of many researchers (Sheikhan *et al*., 2011; Ademyi *et al*., 2012; Pandya *et al*., 2014; Rasheed *et al*., 2014; Wang *et al*., 2015; Harrison *et al*., 2017; Amin, 2018; Alexandra *et al*., 2022). The increase of *h. pylori* among the older patients observed in this study could be attributed to the use of non-steroidal anti-inflammatory drugs (NSAIDS) for the management of pains due to arthritis or rheumatism, and a decrease in mucosal protection due to progressive increase in age (Wang *et al*., 2015; Amin, 2018).

The slight increase in *H. pylori* in single participants and female participants observed in this study could be attributed to differences in stress level, lifestyle and hormonal influences (Rasheed *et al*., 2014; Wang *et al*., 2015). The increase in *H. pylori* among patients who have no formal education could be attributed to low educational background about *H. pylori* and poor sanitary practices. The detection of *H. pylori* mostly among those participants with medical history such as chest pain, heartburn, epigastric pain, stomach ulcer, constipation, and others, supported the findings of many researchers (Sheikhan *et al*., 2011; Ademyi *et al*., 2012; Pandya *et al*., 2014; Harrison *et al*., 2017; Amin, 2018; Alexandra *et al*., 2022).

The detection of *H. pylori* using commercially available immunological kits in this study agrees with the reports of many researchers (Pandya *et al*., 2014; Rasheed *et al*., 2014; Amin, 2018; Kasmi *et al*., 2020; Alexandra *et al*., 2022) but disagrees with the reports of Ferwana *et al*. (2015), Zhou *et al*. (2017) and Nishikawa *et al*. (2018). The study detected more *H. pylori* in stool samples than in blood samples, which was in line with the findings of Amin (2018) and Alexandra *et al*. (2022).

CONCLUSION

The study has shown that H. pylori was significantly detected in blood samples than stool samples using antibody-based test, and sociodemographic factors and medical history of the patients influenced the test

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