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A Comparative Study of the Rate of *Helicobacter pylori* infection Using Three Diagnostic Techniques among Patients in Rivers State

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Approximately 89% of gastric cancers are attributable to *H. pylori* infection, however, only 1 in 5 patients survive longer than 5 years after diagnosis. Evidence has shown that screening and eradication of *H. pylori* in young adults would be cost-effective and could prevent 1 gastric cancer in every 4 to 6 cases. Diagnostic tests used to detect *Helicobacter pylori* are either invasive or noninvasive methods. These tests have varying sensitivity and specificity, having about 90% accuracy in the diagnosis of *H. pylori* infection in clinical practice.
Aim: This study aims at detecting *Helicobacter pylori* using antibody-antigen, stool antigen and polymerase chain reaction (PCR) and to compare the infection rate among the different diagnostic techniques.

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Methodology: This was a cross-sectional study. The participants included attendants to tertiary and private hospitals in Port Harcourt metropolis for medical treatment. Blood and stool samples were collected from one hundred and eighty (180) subjects aged 1 to 60 years and above. Ethical approval for the study was obtained from the Rivers State University Teaching Hospital Ethics Committee. Convenience sampling technique was deployed. Samples were collected from subjects who consented to the study. All samples collected were analyzed in Medical Microbiology Laboratory, Rivers State Teaching Hospital and subsequently, molecular analysis was carried out.
Results: The blood antibodies test (serology) method shows a greater number of positive cases (43.9%) than stool antigen method (8.3%). Also, out of the 75% stool antigen positive samples, subjected to more specific PCR, only 25% were positive. There was a significant difference (*p*-value of <0.001) in sensitivity between antigen and serology, there was also a significant difference (*p*-value of <0.025) between antigen and PCR result outcomes.
Conclusion: The study has shown that there is a notable variation in the diagnosis of *H pylori* among the laboratory techniques used.

Keywords: Helicobacter pylori; infection; diagnostic technique.

1. INTRODUCTION

Globally, approximately 4.4 billion individuals worldwide are estimated to be positive for *Helicobacter. pylori* (*H. pylori)* infection. The highest prevalence rate is in Africa (79.1%), Latin America and the Caribbean (63.4%) and Asia (54.7%). The lowest *H. pylori* prevalence is seen in Northern America at (37.1%) [1].

H. pylori has been identified as a Group 1 carcinogen by the International Agency for Research on cancer and currently is considered necessary but insufficient cause of gastric adenocarcinoma [2,3]. Approximately 89% of all gastric cancers can be attributable to *H. pylori* infection [4], but the prognosis is poor, with only 1 in 5 patients surviving longer than 5 years after diagnosis. The eradication of *H. pylori* has been associated with a reduction of gastric cancer incidence and this benefit is present irrespective of risk group. [5]. Evidence has also shown that the screening and eradication of *H. pylori* in young adults in China would be cost-effective and could help in preventing 1 gastric cancer in every 4 to 6 cases [6]. The development of the H. pylori vaccine has been challenging, with no effective vaccine available on the market yet [7].

Diagnostic tests used to detect *Helicobacter pylori* are either invasive or noninvasive methods. These tests have varying sensitivity and specificity, having about 90% accuracy in the diagnosis of *H. pylori* infection in clinical practice. These tests are currently available, but with different advantages, disadvantages and limitations [8]. Invasive diagnostic methods include endoscopic imaging, histology, rapid urease test, culture, Elisa, and molecular methods, while non-invasive diagnostic tests include urea breath test, stool antigen test and serology. *H. pylori* infection has continued to be a major public health issue worldwide, thus, the need for evaluation of available diagnostic techniques to validate or undermine the efficiency in a resource poor setting.

This study aims at detecting *Helicobacter pylori* using antibody-antigen, Stool antigen and Polymerase Chain Reaction (PCR) and to compare the sensitivity of the different diagnostic techniques. It explored the hypothesis that the antibody-antigen (IgG) technique does not detect *Helicobacter pylori* in human blood samples, the antigen and Polymerase Chain Reaction (PCR) techniques do not detect *Helicobacter pylori* in human stool samples and that there is no significant difference in the diagnostic performances of *Helicobacter pylori* using the different techniques in this study. It seeks to answer the research questions of how precise the indirect method of detecting H. pylori antibodies in human blood samples is, to check if there is a correlation between stool antigen and PCR detection of H. pylori and if there is a significant difference in performances of the different diagnostic techniques.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Rivers State University Teaching Hospital Port Harcourt, Nigeria.
Laboratory analysis was done at Rivers State University Medical Centre Laboratory, located in Nkpolu- Oroworokwo, Port Harcourt. It is a branch of Rivers State University Teaching

Hospital, Port Harcourt and is owned by the government. The molecular analysis was carried out at the Nucleometrix Laboratory in Bayelsa State.

2.2 Study Design

The study is a cross-sectional study. It involved patients who visited the hospital for healthcare services. One hundred and eighty (180) subjects aged 1 to 60 years and above were recruited to participate in the study. The participants had hospital visits for medical treatment to the University of Port Harcourt Teaching Hospital, Rivers State University Teaching Hospital, and Rehoboth Specialist Hospital, all in Port Harcourt Metropolis. Blood samples were collected (180) and stool samples (180), resulting in 360 specimens in total.

2.3 Eligibility Criteria

Persons included in the study were healthy subjects who had no indication of infection or other chronic diseases, persons who visited the hospital with complaints of abdominal pain, heartburn, symptomatic or symptomatic patients aged 1 to 60 years and above.

Persons excluded from the study were those who didn't visit the hospital, those who were immune-compromised and had symptoms of chronic medical conditions like cancer, HIV, and diabetes, and persons who did not consent to the study.

2.4 Sampling Method

The convenience sampling technique was deployed. Socio-demographic information of participants was obtained with the use of a structured questionnaire. Information accessed includes age, place of residence, educational status, antibiotic use, antiretroviral medication and other medical history.

2.5 Sample collection

Samples were collected from subjects who consented to the study. All samples collected were analyzed in the laboratory and subsequently, molecular analysis was carried out.

A total of three hundred and sixty (360) samples were collected, Fecal samples were 180 while blood specimens were 180. The blood sample collected from each participant was five (5 mL) of venous blood, which had serum separated into sterile tubes and stored at a temperature of 2˚C - 8˚C for about 2 days. Analysis for anti-*Helicobacter pylori* antibodies—IgG detection was subsequently conducted. Also, the stool samples from the participants were collected as small pieces of stool (~5 mm to ~150 mm in diameter) into sterile plain screw-capped bottles, these were collected on different days of visits for the outpatients.

2.6 Culture Method

Columbia agar and Mueller Hinton agar were the media used for culture. The color, consistency, and presence of blood/mucus of the stool sample were recorded, each specimen was inoculated onto the agar, which was enriched with sheep blood. It was streaked onto both media plates and incubated under microaerophilic conditions at 37℃ for 2 to 7 days.

Aseptic conditions were maintained in all the steps of specimen collection and inoculation onto culture media, and contamination was minimized.

Pure culture gotten from the mixed growth was sub-cultured onto different fresh media using the appropriate media. The media plates were incubated for further 2 to 7 days at a temperature between 35°C and 37°C. Isolates were identified based on their color, their surface, texture, size of growth, elevation margin, Chemical and biochemical characteristics.

Following culture growth, gram staining and fixing were done. Magnification of x100 magnification with oil immersion objection lens was used to view the slides.

2.7 Serology (Antibody Rapid Test kit), Antigen Rapid Test

A serology (serum antibody IgG) test was done on the blood samples, and an antigen test for Helicobacter pylori was done for the stool sample, with subsequent molecular identification of stool samples that were positive for *H. pylori*.

2.8 Antigen Rapid Test

The detection of *Helicobacter pylori* antigen was carried out with the aid of a one-step cassette-style anti-Hp antigen rapid test kit. In carrying out the test, the specimen was allowed to react with color anti-*H. pylori* monoclonal antibody colloidal

gold conjugates, which were pre-coded on the sample pad of the test. If there were enough *H. pylori* antigens in the specimen, a colored band would form at the test region of the membrane. The test was read after 10 minutes. Two bands on the test region of the cassette in both the control and patient (T) parts of the strip indicate a positive result, while a negative result is indicated when one band shows on the control (C) region. An invalid result is when the band appears on the patient (T) part but doesn't show on the control (C) part.

2.9 Molecular Analysis

Genomic DNA extraction from stool samples was done, followed by DNA quantification, DNA amplification, agarose gel electrophoresis, sequencing, and phylogenetic analysis.

2.10 Genomic DNA Extraction from Stool Samples

Extraction was done using a ZR fecal DNA miniprep extraction kit. A heavy growth of the pure culture of the fungal isolates was suspended in 200 microliters of isotonic buffer into a ZR Bashing Bead Lysis tube, and 750 microliters of lysis solution were added to the tube. The tubes were secured in a bead beater fitted with a 2 ml tube holder assembly and processed at maximum speed for 5 minutes. The ZR bashing bead lysis tube was centrifuged at 10,000 x g for 1 minute. Four hundred (400) microliters of supernatant were transferred to a Zymo-Spin IV spin filter (orange top) in a collection tube and centrifuged at 7000 x g for 1 minute. One thousand two hundred (1200) microliters of fecal DNA binding buffer were added to the filtrate in the collection tubes, bringing the final volume to 1600 microliters. 800 microliters were then transferred to a Zymo-Spin IIC column in a collection tube and centrifuged at 10,000 x g for 1 minute; the flow through was discarded from the collection tube. The remaining volume was transferred to the same Zymo-spin and spun. Two hundred (200) microliters of the DNA Pre-Was buffer were added to the Zymo-spin IIC in a new collection tube and spun at 10,000 x g for 1 minute, followed by the addition of 500 microliters of fungal/bacterial DNA Wash Buffer and centrifuged at 10,000 x g for 1 minute.

The Zymo-spin IIC column was transferred to a clean 1.5 microliters centrifuge tube, 100 microliters of DNA elution buffer were added to the column matrix and centrifuged at 10,000 x *g* for 30 seconds to elute the DNA. The ultra-pure DNA was then stored at -20-degrees Celsius for other downstream reactions.

2.11 DNA Quantification (Nano Dropping)

The extracted genomic DNA was quantified using the Nano drop one thousand spectrophotometers. The equipment was initialized with two ul of sterile distilled water and blanked using normal saline. Two microlitre of the extracted DNA was loaded onto the lower pedestal, the upper pedestal was brought down to contact the extracted DNA on the lower pedestal. The DNA concentration was measured by clicking on the "measure" button.

2.12 Polymerase Chain Reaction (23S rRNA Amplification)

Amplification of the 23S rRNA of *H pylori* was amplified using a nested PCR. The primers Hp23S 1835F: 5′-GGTCTCAGCAAAG AGTCCCT-3′ and Hp23S 2327R: 5′-CCCACCAAGCATT GTCCT-3′ were used for first PCR, and the primers Hp23S 1942F AGGATGCGTCAGTCGCAAGAT and Hp23S 2308R CCTGTGGATAACACAGGCCAGT for second PCR. The amplification was conducted on a master cycler Eppendorf thermal cycler The product was resolved on a 1.5% agarose gel and viewed under UV light.

2.13 Agarose Gel Analysis

1% is equivalent to 1g of Agarose salts dissolved in 100ml of Tris Boric EDTA (TBE). 10 ul of extracted solution was added into wells and a mass ruler DNA ladder into another well for band size measurement. The electrophoretic machine was turned on and allowed to run for 20 minutes.

2.14 Sequencing of 23SrRNA

Sequencing was done using the BigDye Terminator kit on a 3510 ABI sequencer. The sequencing was done at a final volume of 10ul.

2.15 Phylogenetic Analysis

Obtained sequences were edited using the bioinformatics algorithm Trace edit. Similar sequences were downloaded from the National Center for Biotechnology Information (NCBI)

database using BLASTN. These sequences were aligned using ClustalX. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0 (Saitou and Nei, 1987). The bootstrap consensus tree inferred from five hundred (500) replicates (Felsenstein, 1985) is taken to represent the evolutionary history of the taxa analysed. The evolutionary distances were computed using the Jukes-Cantor method (Jukes and Cantor 1969).

2.16 Statistical Analysis

Microsoft Excel was used to capture the data; this was exported to SPSS version 25.0 where descriptive and Chi-square analysis was conducted. The test at a p-value less than 0.05 was significant.

3. RESULTS

Table 1 shows detection of *H. pylori* in stool using antigen and serology techniques. The result shows a significant difference (*p*-value of <0.001) in sensitivity between antigen and serology, with serology showing more sensitivity.

Table 2 shows that subjects who were infected following a stool antigen test were 15 (75%), while those infected following PCR testing were 5 (25%). The result shows a significant difference (*p*-value <0.025).

The bar chart showing the number of persons infected with *H. pylori* against the three analytical technique (serology, antigen and PCR) is represented (Fig. 1).

Table 1. Comparative analysis of *H. pylori* in stool using antigen detection and serology techniques

	No. of Positive (%)	No. of Negative (%)
Antigen	15 (8.3)	165 (91.7)
Serology	79 (43.9)	101 (56.1)
X^2-value	43.57	
p-value	<0.001	

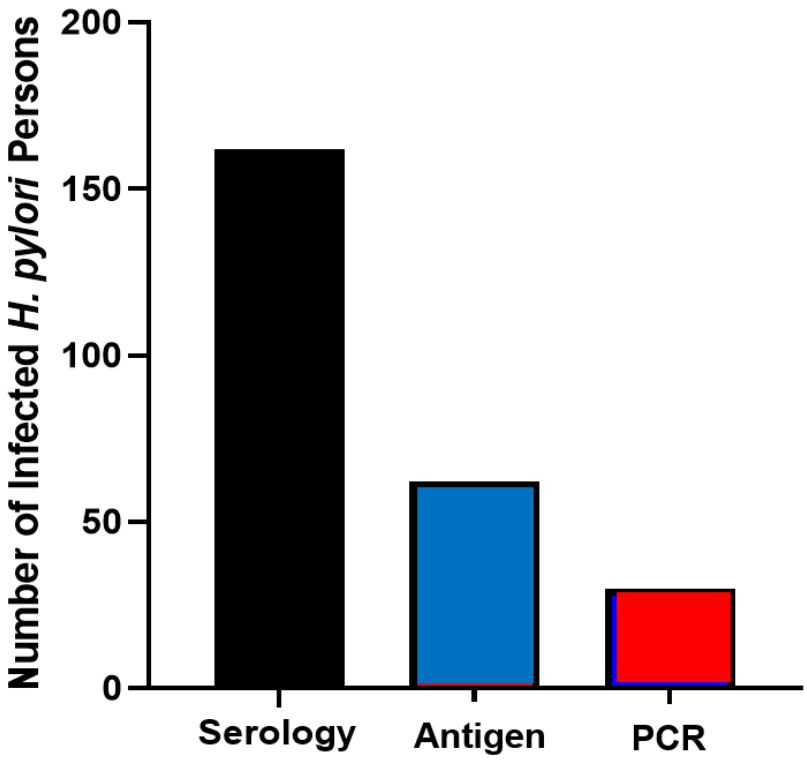

Fig. 1. Bar chart showing comparative analysis of the testing methods

Table 2. Comparison of Analysis of *H. pylori* detection in Stool Using PCR and stool antigen Kit

Technique	No. Positive
Antigen	15 (75%)
PCR	5 (25%)
X^2-value	5.00
p-value	0.025

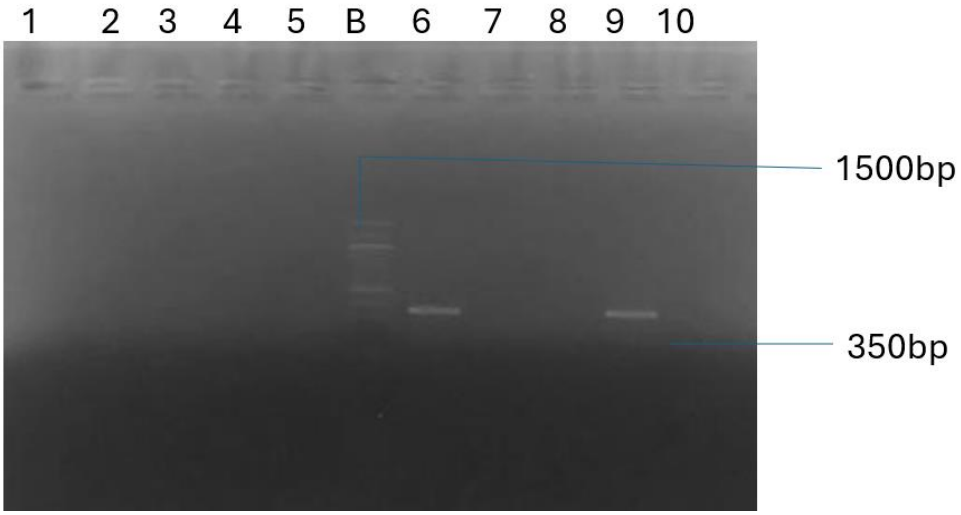

Plate 1. Agarose gel electrophoresis of bacteria isolates
Lanes 6 and 9 represent Hp23s gene bands (350 bp)
Lane B represents the 1500 bp DNA ladder

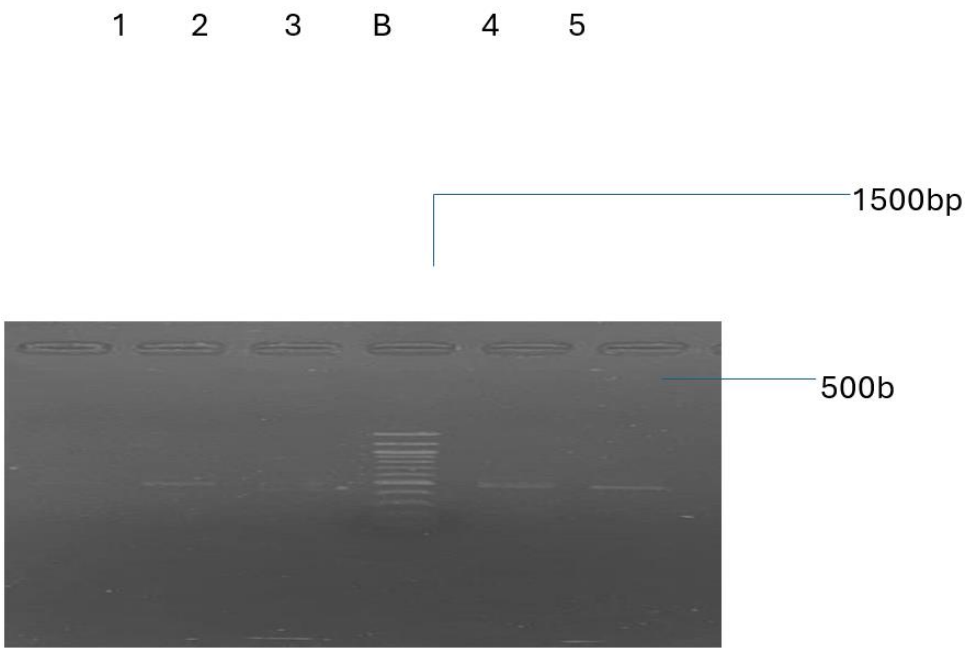

Plate 2. Agarose gel electrophoresis showing HP23 gene of some selected bacteria isolates
Lanes 2, 4 and 5 represent the HP23 gene 500 bp
Lane B represents 100bp DNA ladder of 1500 bp

3.1 The Phylogenetic Tree between the *Helicobacter* and *Campylobacter Species*

The phylogenetic tree showing the association between the *Helicobacter* and *Campylobacter* species is represented in Fig. 2 below.

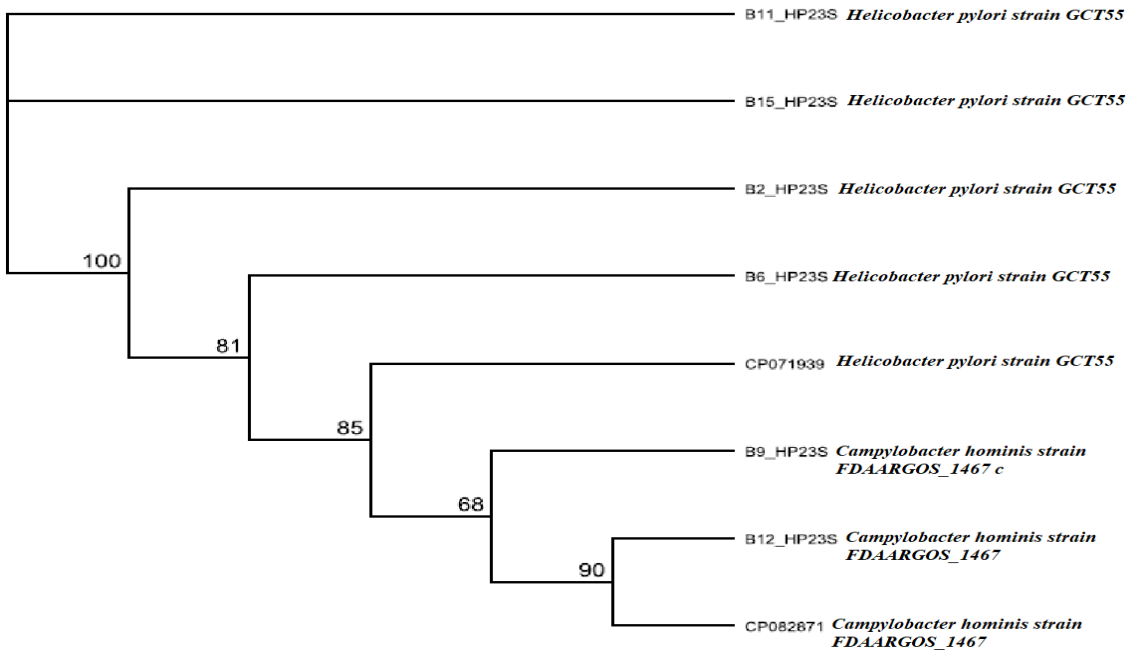

Fig. 2. Phylogenetic tree showing the relationship between the *Helicobacter* and *Campylobacter* species.

4. DISCUSSION

This research was done to compare the different diagnostic methods of non-invasive and invasive techniques, which include serology, stool antigen, and PCR test methods for detection of *H. pylori* infection. The findings of 15 (8.3%) subjects positive to *H. pylori* infection detected by stool antigen test were lower than the work done by Gisbert et al. [9]. The reason could be that more antigens were present in stool. Additionally, it could be due to the advanced technique used in the diagnosis or an insufficient amount of antigen in the stools. Other reasons that might lead to low detection of *H. pylori* with stool antigen could be climate or quality of kit; this agrees with Mahir Gulcan et al. [10], who reported a less positive result (37 out of the 80 subjects), which was comparatively lower than this study.

The blood antibody test (serology) method shows a greater number of positive cases (43.9%) than the stool antigen method (8.3%); this may be due to the experience of past infection. This finding is consistent with that by Arora et al., [11], which showed greater detection of *H. pylori* by serology. In most hospitals in Nigeria and other developing countries, the laboratory depends solely on the detection of antibodies of *H. pylori* as the method of choice in ruling out infection and remains the basis on which treatment is given based on the result of the diagnosis. Out of the 180 samples analyzed, 79 (43.8%) showed positive for serology and 15 (8.3%) positive for stool antigen. One could say that 80% of treated *Helicobacter pylori* cases are false positive if one should rely on the indirect (antibody detection) technique for diagnosis. These false positive results could be due to past infections since the disease is a chronic infection and IgG antibodies formed during the active stages of infection remain in circulation for a period (about 6 months). Secondly, antibodies formed against related gastrointestinal organisms like *Campylobacter jejuni*, *H. caneadi, H. fennallai, C. reseliensis*, and *C. cani* may also contribute to a false positive result for *H. pylori* infection, indicating a low sensitivity of this method. Recall that the phylogenic tree shown relationship between *H pylori* and *Camphylobacter species*, hence this relationship can result to false positive reaction of *H pylori.* So, most of the false positive

results may be attributable to *Camphylobacter species* infection.

On the other hand, out of the 15-stool antigen positive samples, subjected to the more specific PCR, only 5 were positive. This gap could be due to the presence of antigen in the stool from related gastrointestinal organisms such as those of *C. jejuni*.

The serology and stool antigen test (SAT) are test methods for detecting *H. pylori* infection in symptomatic and asymptomatic patients. The stool antigen test method is less expensive, has a sensitivity of 94.1%, a specificity of 95.6%, and an accuracy of 93.8% [12]. The SAT method detects active infection with *H. pylori*, while serology IgG (antibody-antigen) base tests cannot differentiate between active and past *H. pylori* infection and cannot be used for further study with *H. pylori* infection [13-16].

In the detection of *H. pylori* infection with PCR technique, especially when nested PCR was adopted and regarded as the gold standard for *Helicobacter* diagnosis through the construction of specific primers, as in this study, the nested PCR is highly sensitive by excluding false-negative results due to low bacterial count and PCR inhibitor [17]. In Table 2, out of the 15 stool samples positive for the stool antigen test (SAT) that was sent for PCR, only 5 were positive for *H. pylori* infection. This could be because of other related organisms that have a similar genetic property with *H. pylori*, like *C. hominis*, that was detected among the *H. pylori* in the phylogenetic tree.

In general, the gold standard test for the diagnosis of *H. pylori* infection is PCR, though it is expensive and a time-consuming feat for routine diagnostic laboratory testing. However, the use of the direct diagnostic method in stool antigen testing can be recommended for use if PCR is not available instead of antibody detection. The use of this method could save economic wastage, misdiagnosis, and wrong treatment. The overall abuse and misuse of antibiotics is a current problem in the medical industry and has caused multiple drug resistance since the culture method for this organism in the routine laboratory is difficult, and as such, antimicrobial susceptibility testing is unattainable.

This study has been able to add to existing knowledge as it related three diagnostic methods for *Helicobacter pylori* infection with significant variation suggesting that the anti-*Helicobacter pylori* antibody detection kit (indirect test method) is not highly reliable as cross-reaction to similarly circulating antibodies could cause a false positive result.

5. CONCLUSION

Having the PCR technique as the gold standard for *H pylori* infection diagnosis, comparing the diagnostic accuracy of serology and SAT in relation to the gold standard revealed that SAT had better diagnostic accuracy compared to the serology technique which reported high infection rate of 43.9% while SAT recorded 8.3%. The SAT diagnostic outcome was more comparable to the gold standard than the serology technique in *H pylori* investigation.

6. RECOMMENDATION

Antigen stool should be utilized as a laboratory monitoring tool for treatment efficiency rather than the use of antibody- based serological technique. Also, molecular diagnosis should take center stage in the detection and epidemiological surveillance of antibiotic resistance stewardship among *H. pylori* patients.

7. LIMITATION

This study was conducted among 180 subjects between the ages of 1 and 60 who attended tertiary and private hospitals in Port Harcourt metropolis for medical treatment. A similar study carried out with a larger sample in a different age population and location may yield a different outcome. It is in this regard that this research is limited.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

ETHICAL APPROVAL AND CONSENT

Ethical approval for the use of human subjects for this study was obtained from the Rivers State

University Teaching Hospital Ethics Committee after the study proposal was considered, which letter RSUTH/REC/2022189 and dated 24/08/2022. Furthermore, written consent was gotten from subjects who indicated interest to participate in the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Hooi J, Lai W, Ng W, Suen M, Underwood F, Tanyingoh D, Malfertheiner P, Graham D, Wong V, Wu J, Chan F, Sung J, Kaplan G, Ng S. Global Prevalence of *Helicobacter pylori* Infection: Systematic Review and Meta-Analysis. Gastroenterology. 2017;153:420-429.
2. Eslick G, Lim L, Byles J. Association of *Helicobacter Pylori* Infection with Gastric Carcinoma: A Meta-Analysis. American Journal of Gastroenterology. 2016;94: 2373-2379.
3. Huang, J, Sridhar S, Hunt, R. Role of *Helicobacter pylori* infection and non-steroidal anti-inflammatory drugs in peptic ulcer disease: A meta-analysis. Lancet. 2002:359:14-22.
4. DM, Forman D, Bray F. Cancer incidence and mortality worldwide. IARC cancer base. International Agency for Research on Cancer. GLOBOCAN VI.O. 2012; 11.

   Available:http//globacom.iarcfr
5. Kosuneu T, Pukkala E, Sarna S, Seppala K, Aroma A, Knekt P. Gastric cancers in Finnish patients after cure of *Helicobacter pylori* Infection: A cohort study. International Journal of Cancer. 2011; (128):433-439.
6. Yamaoka K, Oka M, Chata H. Eradication of *Helicobacter pylori* prevent cancer development in subjects with mild gastric atrophy identified by serum pepsinogen levels. International Journal of Cancer, 2009;125(11):2697-2703.
7. Lu B, Li M. Helicobacter pylori eradication for preventing gastric cancer. World Journal Gastroenterology, 2014;20:5660-5665.
8. Cardos A, Maghiar A, Zaha D, 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; 12:508.

   DOI: 10.3390
9. Gisbert J, Alcedo J, Amador J, Bujanda L, Calvet X, Castro-Fernández M, Fernández-Salazar L, Gené E, Lanas Á, Lucendo A. Conferencia Española de Consenso sobre el tratamiento de la infección por *Helicobacter pylori*. Review Española Enferm. Digestive. 2021;113: 740–763.
10. Balan K, Subbukesavaraja. Comparative study of noninvasive methods for diagnosis of *Helicobacter pylori* in humans. Scholars Academic Journal of Biosciences. 2013; 1(1):14-17.
11. Arora S, Bisen G, Budhiraja R. Mucoadhesive and Mucopenetrating Delivery Systems for Eradication of *Helicobacter pylori*. Asian Journal of Pharmaceutic. 2012;6:18-30
12. Choi J, Kim C, Kim D, Chung S, Song J, Kang J, Yang J, Park M, Kim Y, Yim J. Prospective evaluation of a new stool antigen test for the detection of *Helicobacter pylori*, in comparison with histology, rapid urease test, (13)C-urea breath test, and serology. Journal Gastroenterology Hepatology. 2011;26: 1053–1059.
13. Makristathis A, Hirschl A, Mégraud F, Bessède E. Review: Diagnosis of *Helicobacter pylori* infection Helicobacter. 2019;24:12641.
14. Abadi A. Diagnosis of *Helicobacter pylori* using invasive and noninvasive approaches Journal Pathogenesis. 2018; 9064952:1–13.
15. Calik Z, Karamese M, Acar O, Aksak Karamese S, Dicle Y, Albayrak F. Investigation of *Helicobacter pylori* antigen in stool samples of patients with upper gastrointestinal complaints. Brazil Journal Microbiology. 2016;47: 167–171.
16. Goh K, Chan W, Shiota S, Yamaoka Y. Epidemiology of *Helicobacter pylori* infection and Public Health Implications. Helicobacter. 2011;16:1-9.

17. Patel S, Pratap C, Jain A, Gulati A, Nath G. Diagnosis of *Helicobacter pylori*: What should be the gold standard? World Journal of Gastroenterology. 2014;20(36): 12847-12859.

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