European Journal of Health Sciences (EJHS)



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Comparative Assessment of Current Serological Methods against the Conventional in the Diagnosis of Helicobacter pylori Infections in Suspected Peptic Ulcer Patients Attending Health Facilities in Lafia, Nigeria.

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Abstract

Purpose: *Helicobacter pylori* has been implicated in most severe cases of peptic ulcer. Despite this development, the diagnosis of this infection has been a major challenge due to the difficulty encountered during isolation using the conventional culture method. Presently, most health facilities in rural communities in Nigeria still adopt the culture method as the gold standard technique for the diagnosis of *H. pylori* infections. The need for the introduction of more accurate, robust and rapid diagnostic techniques is therefore imperative. This study was carried out to compare the overall performance of two diagnostic methods in the assessment of *Helicobacter pylori* infection status among peptic ulcer suspected patients attending health facilities in Lafia, Nigeria, using stool antigen immunoassay test and blood antibody test methods.

Methodology: A total of 180 patients with peptic ulcer symptoms attending three health facilities (80 from DASH and 50 each from Jafamek Diagnostic Centre and Haske Hospital) were recruited by designated health workers through random selection using non-probability and convenience sampling. The blood and stool samples of each participant were screened using *H. pylori* antibody/ antigen test strips (Azure Biotech Inc). The stool samples were cultured on Brain Heart Infusion agar (Oxoid, UK) and the result used as a gold standard in this study. Data obtained were presented as frequencies and association between test methods analysed using contingency chi-square test and Cohen kappa statistics.

Results: The outcome of the study showed that out of the 180 participants screened, 51 (28.33%) were positive using the culture method (CM), 111 (61.67%) were reactive for *Helicobacter pylori* blood antibody test (BAB), while 86 (47.78%) were positive for *Helicobacter pylori* stool antigen test (SAG). The sensitivity and specificity of the two methods were recorded as 74.50%, 44.20% and 70.60%, 61.20% for BAB and SAG respectively. The level of agreement according to the value of kappa was found to be *poor* with BAB but *fair* with SAG.

Conclusion: Based on the findings of this study, the overall prevalence of *H. pylori* infection among the patients was relatively low compared to values obtained from other areas in Nigeria. Also, the stool antigen analytical method had the highest diagnostic accuracy compared to the serum antibody and culture techniques.

Recommendation: The stool antigen method is considered the most effective in the diagnosis of *Helicobacter pylori* infections in the study community and should therefore be used regularly as first choice option.

Keywords: Helicobacter pylori, diagnosis, culture, stool antigen, blood antibody.



1.0 Introduction

Over the ancient times, spiral, coiled micro-organisms or 'spirochetes' were seen occasionally in gastric specimens of humans and other carnivorous animals including dogs (Akada *et al.*, 2000). As these gastric organisms were generally not culturable, it was not possible to be identified until 1982 when Barry Marshall and colleagues in Perth (Western Australia), were able to culture a small curved s-shaped bacillus observed microscopically in the antral biopsy material from individuals with gastritis and gastric ulcers (Warren, 1983). This finding provided the background for a fast growing area of microbiology with the recognition of a diversity of new species with unique microbiological properties and disease associations (Argent *et al.*, 2002).

Helicobacter pylori is a gram negative bacterium that belongs to the family *Helicobacteraceae* (Aviles-Jimenez *et al.*, 2004). It is a curved shaped bacterium (Tomb *et al.*, 1997) understood to be closely associated to a pair of severe stomach disorders, ulcers and stomach cancer (Omosor *et al.*, 2017). *Helicobacter pylori* infection has been strongly associated with many gastroduodenal diseases such as peptic ulcer, gastritis, mucosa-associated lymphoid tissue lymphoma and non-cardiac gastric cancer (Yang & Rothman, 2004; Reshetnyak *et al.*, 2021). It is able to grow in the human stomach regardless of the highly acidic conditions that make it unfavourable for other microorganisms to survive (Yang *et al.*, 2008). *H. pylori* secretes an enzyme which converts urea to ammonia thereby reducing the potency of stomach acid to affect its growth (Atherton *et al.*, 1995).

It is estimated that half of the world's population are carriers of this bacterium (Hunt *et al.*, 2011). The prevalence of *H. pylori* infection is 30 - 50% in developed countries and 70 - 90% in developing countries (Cano-Contreras *et al.*, 2018). Research has shown that *H. pylori* can weaken the protective coating of the stomach. When this occur, digestive juices are more likely to irritate the stomach lining, resulting in a stomach ulcer. In serious cases, stomach acid may erode the stomach lining causing a perforation (Hunt *et al.*, 2011). It has long been known that *H. pylori* were a key factor in stomach cancer. The World Health Organization has classified the bacterium as a group I carcinogen for stomach cancer (Adeniyi *et al.*, 2012). *H. pylori* exist in the stomach of millions of people in asymptomatic state hence does not develop stomach cancer or an ulcer (Gramstrom *et al.*, 1997; Zamani *et al.*, 2018). However, others may manifest a variety of digestive disorders when their immune systems are compromised (Gold, 1996).

Several researchers reported higher increase of *H. pylori* infection in developing countries relative to developed countries (Demiray *et al.*, 2006). According to few studies in Iraq, *H. pylori* prevalence ranged from 11.3 to 71.3% (Majeed & Khashnaw, 2020; Hussein *et al.*, 2021). High prevalence of *Helicobacter pylori* infection from other countries including Nigeria have been reported. Bashir and Ali (2009) reported an *H. pylori* prevalence of 81% in Kano, Malu *et al.* (2004) reported a prevalence of 87% in Jos while Aboderin *et al.* (2007) reported 73% in Ile-Ife, South-West Nigeria. Studies from other African countries reported similar prevalence rates such as 91.7% in Egypt (Galal *et al.*, 2019) and 75.4% in Ghana (Bako and Darko, 1996).

Presently, most health facilities in rural communities in Nigeria still adopt the culture method as the gold standard technique for diagnosing of *H. Pylori* infections. There is also a paucity of data on the validity of different diagnostic techniques in areas where other techniques are being introduced. Consequently, the epidemiological information on the prevalence of *H. pylori* in most part of Nigeria is scarce. This study was therefore aimed at investigating the infection status of *Helicobacter pylori* among suspected peptic ulcer patients attending



health facilities in Lafia, Nasarawa State, Nigeria, using stool antigen immunoassay test and blood antibody test against the culture method (conventional method) which serves as the gold standard.

2.0 Methodology

2.1 Study Area

The study area comprised of health facility centres within Lafia, Nasarawa State, Nigeria. Lafia is a semi-urban town in North Central Nigeria and the capital city of Nasarawa State. It has a population of approximately 330,712 inhabitants whose occupation are mainly farming, business and artisans. There is one (1) tertiary health facility and few primary and secondary health centres. The health facilities used for this research were Dalhatu Araf Specialist Hospital (DASH), Jafamek Medical Laboratory and Diagnostic Centre and Haske Hospital.

2.2 Determination of Sample Size

The sample size (n) was estimated using the formula described by Thrusfield (2005) which states:

 $n = (1.96)^2 pq/d^2$

Where;

n = required sample size,

p = proportion of a similar population having *H. pylori* infection from previous study,

q = 1 - p

d = the degree of precision

For the calculation (at 95% confidence interval):

p value = 0.865 based on a prevalence rate of 86.5% from previous study by Ejilude et *al*. (2000)

Desired precision (d) set at 0.05 was used to determine the minimum sample size required, giving a total sample size of 180.

2.3 Experimental Design

A total of 180 suspected peptic ulcer patients attending three health facilities in Lafia, Nasarawa State were used for this study. Eighty (80) blood and stool samples were collected from the subjects (each giving a stool and blood sample) attending DASH while 50 samples each of blood and stool were collected from Jafamek Diagnostic Centre and Haske Hospital. Patients were randomly selected by non-probability, convenience sampling and were recruited by designated health workers.

2.4 Ethical Consideration

Ethical approval for the study was obtained from the Nasarawa State Ministry of Health and Ethical Committee of the various health facilities.

2.5 Eligibility of Subjects:

a) Inclusion criteria

Suspected patients with signs and symptoms of peptic/gastric ulcer attending these health facilities were included.



b) Exclusion criteria:

Patients with symptoms undergoing antibiotics treatment for *H. pylori* infection and those not related to peptic/gastric ulcer were not enrolled. Antibiotics have been reported to intervene with the accuracy of *H. pylori* detection and researchers have been advised to take biopsies for diagnosis at least 3 months after the patient ceases the administration of antibiotics (Mayo Foundation for Medical Education and Research, 2022).

c) Consent:

Informed consent was obtained from each willing participant whose blood and stool specimen was used for the study. The objectives, benefits and procedure for the study was made very clear to the participants and they were assured of the confidentiality and voluntariness associated with their participation.

2.6 Sample Collection

Both blood and stool samples were collected from each participant.

2.6.1 Venous blood collection:

Five millilitres (5ml) of venous blood sample was collected from each patient into plain blood bottles. The blood samples were transported to the laboratory and processed within 2 hours. The serum was separated by centrifugation of the blood sample at 3000 rpm for 5 min. and transferred immediately into sterile tubes which were then stored at $2^{\circ}C - 8^{\circ}C$ for a maximum of 3 days prior to analysis. For long term storage, samples were kept below -20°C.

2.6.2 Stool sample collection:

Self-collected stool samples were requested from each participant. They were given sterile leak-proof single use universal bottle with a screw-capped lid, as well as instruction on how to collect their stool samples aseptically in private.

The stool samples were transported to the laboratory and processed within 2 hours. For long term storage, samples were kept at 4°C in the refrigerator if delay was also expected (Ejilude *et al.*, 2000).

2.7 Sample Analyses

Helicobacter pylori infection was detected in the patients' samples using three methods: the stool antigen immunoassay test method, blood antibody and culture method.

2.7.1 Blood antibody test

All stored sera samples obtained from the suspected patients were screened using the Accu-Bind antibody test device (Diasure diagnostic test, Azure Biotech Inc.) for the detection of the presence of IgG antibodies to *H. pylori* in the samples (Ejilude *et al.*, 2000).

2.7.2 Stool antigen immunoassay test

Frozen samples were completely thawed and mixed thoroughly prior to analysis. Small piece of stool (5mg) was transferred into 1 ml of Sample Treatment Solution (STS) in a test tube and mixed vigorously. The sample was screened using *Helicobacter pylori* antigen rapid test device for faecal sample (Diasure rapid diagnostic test Azure Biotech inc.) which is a lateral flow chromatographic immunoassay for the detection of *H. pylori* antigen in human stool samples (Ejilude *et al.*, 2000).



2.7.3 Culture and Isolation of Helicobacter pylori

This was performed based on the method described by Amin *et al.* (2019). Stool samples were streaked on Brain Heart Infusion agar (Oxoid, UK) (5% fecal bovine serum, 7% horse blood and antibiotics such as nystatin, nalidixic acid, and vancomycin) and incubated at 37°C for 3-5 days under microaerophilic conditions with an oxygen content of $\leq 0.5\%$. Growths were further sub-cultured to have a pure isolate of *H. pylori*. Gram staining and hanging drop motility assessment were carried out for confirmative identification.

2.8 Determination of specificity and sensitivity of blood antibody and stool antigen methods.

Sensitivity and specificity are statistics that are most often used to describe the utility of diagnostic tests in clinical settings. Specificity and sensitivity of each analytical method in relation to the culture method will be calculated as follows:

Specificity = $R'_{ANA}R'_{CM} / R'_{ANA}R'_{CM} + R_{ANA}R'_{CM}$

Where: $R'_{ANA}R'_{CM}$ = Number of cases non-reactive for both analytical and culture methods.

 $R_{ANA}R'_{CM}$ = Number of cases reactive for analytical and non-reactive for culture methods.

 $Sensitivity = R_{ANA}R_{CM} / R_{ANA}R_{CM} + R'_{ANA}R_{CM}$

Where: $R_{ANA}R_{CM}$ = Number of cases reactive for both analytical and culture methods.

 $R'_{ANA}R_{CM}$ = Number of cases non-reactive for analytical and reactive for culture methods.

2.9 Statistical Analyses

Results were presented as frequencies and percentages. Comparison of the prevalence of *H. pylori* was expressed in percentage. Contingency Chi square tests were used to study the association between test methods. Cohen's Kappa statistics was used to measure concordance between the test methods (McHugh, 2012). The collected data were analysed using the Statistical Package for Social Sciences version 26 (IBM SPSS Inc., IL, Chicago, USA) for Windows. Two-tailed P¬-values of <0.05 were considered to be significant.

3.0 Results

Table 1 shows the prevalence of *Helicobacter pylori* among suspected patients attending various health facilities using the three analytical methods namely culture (CM), blood antibody (BAB) and stool antigen immunoassay (SAG) tests. The result revealed that 51(28.33%), 111(61.67%) and 86(45.93%) were reactive for *H. pylori* infection by culture, blood antibody and stool antigen immunoassay test methods respectively. The result also revealed a significant difference (p<0.001) in the prevalence values using the three analytical methods. Out of a total of 540 samples analysed, the three methods revealed an overall prevalence of 45.93% of *H. pylori* infection with 248 reactive cases.



Table 1:	Prevalence	of	Helicobacter	pylori	among	patients	according	to	analytical
methods									

Test Method	Total no. of Samples N	No of reactive Samples n (%)	<i>x</i> ²	Р
СМ	180	51 (28.33)	40.64	< 0.001
BAB	180	111 (61.67)		
SAG	180	86 (47.78)		
Total	540	248 (45.93)		

CM= *Culture method*; *BAB*= *Blood antibody method*; *SAG*= *Stool antigen method*.

The percentage occurrence of *H. pylori* infection from suspected patients using the three analytical methods and their combinations is shown in figure 1. Highest value of 27% was obtained for subjects that were only positive for blood antibody method while the least (5%) was obtained for those only positive for the culture method. There was a significant difference (p<0.001) in the percentage values of subjects positive to only one specific method. However, samples positive for both blood antibody and stool antigen methods also had a high percentage value of 25% and a similar value of 22% was obtained for samples positive for the three analytical methods.



Figure 1: Percentage occurrence of *H. pylori* from suspected patients using the three analytical methods

Table 2 shows the comparison of positive cases based on combinations of analytical methods. The result revealed a significant difference in the prevalence values (p<0.001, <0.026, <0.001 and <0.001) in all analytical combinations (CM vs SAG, CM vs BAB, SAG vs BAB, and CM vs SAG vs BAB respectively). The result showed that combinations of both the stool antigen test and the blood antibody test produced significantly higher values (p<0.001) compared to other combinations.



	Total no. of samples	No. of reactive samples		
	Ν	n (%)	\mathbf{x}^2	P-Value
CM vs SAG	180	36 (20.00)	14.84	< 0.001
CM vs BAB	180	38 (21.11)	4.97	0.026
SAGvs BAB	180	65 (36.11)	13.49	< 0.001
CM vs SAG vs BAB	180	30 (16.67)	15.91	< 0.001

Table 2: Comparison of reactive cases based on different combinations of analytical	
methods.	

CM vs *SAG* = *Culture method vs Stool Antigen method; CM vs BAB* = *Culture method vs Blood Antibody method*

Table 3 shows the association in the prevalence values of reactive and non-reactive cases using blood antibody and stool antigen tests in relation to the culture (conventional) method. Cases reactive to both blood antibody and culture methods had highest prevalence of 74.50% while cases non-reactive to both methods had 44.20%. Cases with different reactions had 55.8% (reactive for blood antibody test and non-reactive for culture method) and 25.5% (non-reactive for blood antibody test and reactive for culture method). Cases with similar positive reaction for both stool antigen and culture methods were 36 (70.60%) while similar non-reactive cases were obtained in 61.20% of the subjects. Different reactions were obtained in 38.80% (reactive for stool antigen test and non-reactive for culture method) and 29.40% (non-reactive for stool antigen test and reactive for culture method) of the cases analysed. The p value in both associations (p=0.020 and p <0.001 for blood antibody and stool antigen respectively) indicated that there is significant relationship between the conventional method and the two analytical methods with stool antigen test having higher association compared to the blood antibody test method.

		Culture Method				
		Non-reactive (R ['] _{CM})	Reactive (R _{CM})	Total	-	
	Test-outcome	n (%)	n (%)	n (%)	<i>x</i> ²	Р
	Non-reactive (R' _{BAB})	57 (44.20)	12 (25.50)	69 (38.30)	5.38	0.020
Blood Antibody test	Reactive (R _{BAB})	72 (55.80)	39 (74.50)	111(61.70)		
	Total	129 (100)	51 (100)			
	Non-reactive(R' _{SAG})	79 (61.20)	15 (29.40)	94 (52.2)	14.48	<0.001
Stool antigen test	Reactive(R _{SAG})	50 (38.80)	36 (70.60)	86 (47.80)		
	Total	129 (100)	51 (100)	180 (100)		

 Table 3: Association in the prevalence values of reactive and non-reactive cases using the blood antibody and stool antigen tests in relation to the culture method.



 R'_{CM} – Non-reactive cases using culture; R_{CM} - Reactive cases using culture; R'_{BAB} – Non-reactive cases using blood antibody method; R_{BAB} – Reactive cases using blood antibody method; R'_{SAG} – Non-reactive cases using stool antigen; R_{SAG} - Reactive cases using stool antigen

Table 4 showed the sensitivity and specificity assessment of both blood antibody and stool antigen analytical methods in relation to the culture method. The blood antibody method had specificity and sensitivity values of 44.20% and 74.50% respectively in relation to the culture method. Specificity and sensitivity values for the stool antigen method in relation with the culture method were 61.20% and 70.60% respectively.

RBABR'CMSpecificity7244.20	(%)
72 44.20	
R ['] _{BAB} R _{CM} Sensitivity	(%)
13 74.50	
R _{SAG} R' _{CM} Specificity	(%)
50 61.20	
R' _{SAG} R _{CM} Sensitivity	(%)
15 70.60	
	1374.50RsagR'CMSpecificity5061.20R'sagRCMSensitivity

 Table 4: Determination of sensitivity and specificity of blood antibody and stool antigen analytical methods

 R'_{CM} – Non-reactive cases using culture, R_{CM} - Reactive cases using culture, R'_{BAB} – Non-reactive cases using blood antibody method, R_{BAB} – Reactive cases using blood antibody method, R'_{SAG} – Non-reactive cases using stool antigen, R_{SAG} - Reactive cases using stool antigen

Table 5 shows the inter-rater reliability and level of concordance according to the value of Kappa of the two diagnostic methods and the conventional method (gold standard). In comparison between culture versus blood antibody methods, the result revealed that the level of concordance between these methods was *poor* according to Kappa value of 0.26. However, in the comparison between culture versus stool antigen methods, the level of concordance between these methods was *poor* according to Kappa value of 0.26. However, in the comparison between culture versus stool antigen methods, the level of concordance between these methods was *fair* based on the Kappa value of 0.14.

Test Methods	Kappa	p –Value	IRR	Sensitivity	Specificity
CM vs BAB	0.14	< 0.020	Poor	74.50	44.20
CM vs SAG	0.26	< 0.001	Fair	70.60	61.20

Table 5: Level of Agreement according to value of Kappa

CM = Culture method; BAB = Blood antibody method; SAG = Stool antigen method, IRR= inter-rater reliability; Level of agreement according to value of Kappa: Poor: <0.20; Fair: 0.21 - 0.40; Moderate: 0.41 - 0.60; Good: 0.61 - 0.80; Very good: 0.81 - 1.00.



4.0 Discussion

Helicobacter pylori is a very common infection in developing countries, therefore a low-cost, rapid diagnostic technique may be useful for the management of *H. pylori* infection in both children and adults from these developing regions. Nigeria is one of the developing countries with rising incidents of gastritis and duodenal ulcers, as well as increasing prevalence of *H. pylori* infection hence the need to re-appraise the performances of different cost-effective, easy to use methods in diagnosis of *H. pylori* infection in the general population. In this study, an overall prevalence of 45.93% was reported. This was however lower than 81% obtained by Bashir and Ali (2009) in Kano, 87% obtained by Malu *et al.* (2004) in Jos and 73% reported by Aboderin *et al.* (2007) in Ile-Ife, all in Nigeria. These differences may be due to the difference in the diagnostic techniques applied and the clinical statues of the patients enrolled in the studies. Hussein *et al.* (2021) reported an overall difference in the diagnostic method applied.

The overall *H. pylori* reactive rate in this study was higher in the blood antibody test (61.67%) compared to the stool antigen test (47.78%) and culture (28.33%) although culture method was used as a gold standard to compare the two methods. It is well known that the culture method is not a regular process in early assessment (Samuel *et al.*, 2000) and mostly not used for routine check-ups because of the demanding character of this bacterium (Taj *et al.*, 2003). Namavar *et al.* (2003) also stated that *H. pylori* could be cultured from stool specimens but colonies have to be further categorized by other methods due to the occurrence and growth of several other bacteria and especially microorganisms phenotypically comparable to *H. pylori*. In a study by Kelly *et al.* (1994), among presumptive *H. pylori* isolates cultured from fresh stool specimens, only 12 out of the 25 subjects were found to be *H. pylori* infected patients. In similarity with this study, the authenticity of these *H. pylori* isolates has not been established, as they were not tested for oxidase activity and their identity was not established by sequence analysis of the 16s rRNA gene. Aje *et al.* (2010) also reported lesser reactive cases in stool antigen tests (48.9%) compared to the serology tests (67.4%).

This may be attributed to the fact that *H. pylori* stool antigen test is a more accurate indicator of active disease compared to serology test, which may not measure active disease conditions (Omosor *et al.*, 2017). *H. pylori* stool antigen test has been shown to be rapid, easy to use and differentiates between active and latent infection; whereas, serology only detects exposure and are not suitable to diagnose active infection or follow-up of eradication because of its low accuracy (Bako & Darko, 1996). Similarly, Hussein *et al.* (2021) working with Patients in Iraq reported an overall performance of the stool antigen test over the blood antibody and culture tests. However, the result of the stool antigen test method in this study was relatively lower than that of a recent study by Galal *et al.*(2019) in Egypt who reported 64.6% occurrence rate. The stool antigen test in this study was seen to have a sensitivity and specificity of 70.60% and 61.20% whereas the blood antibody has sensitivity and specificity of 74.5% and 44.22% respectively. This result is in agreement with a previous study by Gisbert & Pajares (2004) who reported stool antigen test result with sensitivity and specificity of 88.5% and 99% whereas the serum antibody had sensitivity and specificity of 93.8% and 98% in adults. It is observed that both studies show high specificity in stool antigen and high sensitivity in blood antibody test.

A closer look at the level of agreement between the culture method and the other two methods (blood antibody and stool antigen test) indicates that the blood antibody test gave a *poor* representation of the true prevalence whereas the stool antigen test gave a *fair* representation of the true prevalence. This is in contrast with the findings of Aje *et al.* (2010), whose result



revealed that the stool antigen test and IgG serology can give a fair representation of the true prevalence in dyspeptics in Nigeria using histology as the gold standard.

In this study, some of the participants tested positive to the serum antibody, but negative for stool antigen, while some tested positive for both serum antibody and stool antigen. Still, some tested negative for both serum antibody and stool antigen. The implication of this may be that those that tested positive for only serum antibody and not stool antigen must have been treated and the reaction is due to previous exposure to *H. pylori* (past infection), hence the reason for the presence of antibody to *H. pylori* in their serum. Those that were reactive to both serum antibody and stool antigen are having recent infection while those that were negative to both serum antibody and stool antigen are susceptible to *H. pylori* infection.

5.0 Conclusion

The results obtained in this study prove that *H. pylori* infection is present among patients attending health facilities in Nasarawa State with a prevalence rate of 28.33%, 61.67% and 47.78% using culture, blood antibody and stool antigen tests respectively. The result also revealed the effectiveness of the stool antigen immunoassay test method in comparison to the blood antibody test.

6.0 Recommendations

The stool antigen method is considered the most effective in the diagnosis of *Helicobacter pylori* infections in the study community and should therefore be used regularly as first choice option. Regular screening for *Helicobacter pylori* infection among symptomatic and asymptomatic individuals in the study community is imperative to effectively control its spread.

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