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Original Article

Evaluation Of Diagnostic Methods For Detection And Isolation Of *Helicobacter Pylori* In Gastric Biopsy And Stool Specimens Of Dyspeptic Nigerians

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Summary

The current trend in the diagnosis of *H*. *pylori* infection is to move from invasive diagnostic methods to a non – invasive method. This study was carried out to compare the available methods of diagnosis of *Helicobacter pylori* infections. Stomach biopsy, stool, and blood samples of participants were subjected to analysis for *H.pylori* infection, using Gram stain, Polymerase Chain Reaction (PCR) , Urease test, serology, Stool Antigen test, stool and biopsy cultures. Of the 320 patients studied, 198 (61.8%) were *H. pylori* positive by direct Gram's stain and urease (CLO) positivity was found in 134 (41.8%). A total of 272 (85%) were seropositive for *H. pylori* 1gG, while *H. pylori* was isolated by culture from biopsies of 67 (20.9%) and stools of 40 (12.5%) patients. *Helicobacter pylori* antigen (HpSA) was also detected in 234 (73.1%) patients and while PCR detected positivity in 162 (50.6%) of the participants. The results from this study showed 100% sensitivity using serology and HpSA. Using stool culture, the positive predictive value and specificity was also 100%. Serology showed the highest negative predictive value (100%) while stool has the highest false negative rate of 60.9%. A combination of HpSA, stool culture and serology was found suitable for the detection of *H. pylori* infection in our environment and no single test was found to be best diagnostic method.

Keywords: *Helicobacter pylori*, diagnostic methods, biopsy, stool, dyspeptic.

Introduction

Helicobacter pylori is the commonest bacterial infection worldwide [1,2,3]. Thus, there is a considerable interest in diagnostic methods for *Helicobacter pylori* infection both before and after treatment [4].

Detection of *Helicobacter pylori* infection in patients undergoing upper gastrointestinal

endoscopy is required as evidence to start eradication treatment in those with peptic ulcer disease and non-ulcer dyspepsia[5]. There are several reliable methods for detecting *Helicobacter pylori* but all have the disadvantage that include cost and time required to yield definitive result [6,7].

In general, all diagnostic tests have a 5 – 10% false positive or false negative rate and only by combining diagnostic tests do we come close to a true gold standard[8]. Most of the time, investigators do not always agree on the best standards against which to validate a test. There has been much interest in tests that provide information over and above merely the presence of *Helicobacter pylori*, for example tests that indicate whether antimicrobial resistance or certain virulence factors are present. There are, however, many pitfalls. In the Western world only 5 – 10% of individuals are infected with two or more different *H. pylori* strains. In the developing world, where the prevalence of overall infection is higher, multiple strain infection are common[9]. There are large differences in strains around the world[9] and, because of this micro diversity, molecular techniques may not be suitable for global use[10]. Diagnostic methods for *Helicobacter pylori* infection may be invasive or non-invasive[4,11].

To confirm the presence of *Helicobacter pylori* infection, mucosal biopsy specimens obtained during upper gastrointestinal endoscopy could be cultured, examined histologically or used for urease test and polymerase chain reaction. Non-invasion tests like urea breath test, Helicobacter pylori Stool Antigen test (HpSA) and serology are more convenient.

The *H. pylori* stool assay and the Helicoblot, immunoblot kit are two new non-invasive tests that are now commercially available[12]. Most of these tests have sensitivities and specificities greater than 90% [7,12,13] but there is a lack of agreement on any single test as the gold standard[7]. Most investigators classified patients as *H. pylori* positive on the basis of a positive culture or a combination of positive histology and urease test or any two positive tests or on the "a posteriori" based empirical interpretation of results described by [12,13]. The proposed independent gold standard by the stipulation that any positive test had to be confirmed by at least one other positive test is arbitrary, but satisfactory[7]. A variety of tests are now available to diagnose *H. pylori* infection. The choice of diagnostic test for assessing *H. pylori* status is dictated by clinical considerations, the need for endoscopy, reliability, specificity, sensitivity, cost, local access and expertise. As a general rule, physicians should choose a test that has the best accuracy for the level of testing expertise available.

However, the "gold standard" for the diagnosis of *H. pylori* infection is growth of the pathogen in cultures, thus requiring gastric biopsy specimens obtained by invasive upper gastrointestinal endoscopy[15]. This proves a bit difficult among children and younger patients with dyspeptic symptoms. Therefore,

noninvasive tests are of major importance for evaluation of such patient's status most importantly after treatment. This study was carried out to compare the available methods of diagnosis of *Helicobacter pylori* infection using both the invasive and non invasive methods/samples.

A variety of tests are now available to diagnose *H. pylori* infection. Therefore, the diagnostic methods for *H. pylori* infection are classified under methods requiring endoscopy such as histological examination of gastric tissue, bacterial culture, rapid urease testing, use of DNA probes and PCR analysis on gastric biopsies / tissue. These methods incur expenses and a risk and slight complication due to the procedure. On the other hand Urea breath tests, serology, gastric juice PCR, urinary excretion of [^{15}N] ammonia and EIA for *H. pylori* antigen in stool (HpSA) and stool culture are non-invasive tests that do not require endoscopy. Other assays include HpSA-EIA (*H. pylori* Stool Antigen – Enzyme Immunosorbent Assay), collection of blood or urine to measure substrate metabolism by *H. pylori* [16], similarly, the measurement of ^{14}C in the urine has been reported to accurately reflect *H. pylori* status[17]. However, none of these methods has been completely standardized, and they do not confer significant advantage over the previously described diagnostic methods.

Existing antimicrobial therapy is not always completely effective against *H. pylori* infection. As a result clinicians may wish to determine if the patient has been cured of *H. pylori* after treatment. Most of the test for the initial diagnosis of *H. pylori* can also be used for post treatment diagnosis. In general, evaluating for a cure of *H. pylori* with tests that assess the actual bacterial load (UBT and biopsy methods) should not be performed less than 4 weeks after therapy[18]. This is because up to 4 weeks after treatment that ultimately is shown to have failed, it may not be possible to detect *H. pylori* due to 'suppression' or reduction of bacterial load. This phenomenon has been termed 'clearance'. A prolonged time is (more than 4 weeks) required to allow suppressed populations to re-grow to their original densities in the mucosa.

Materials and Methods

Study Population

Three hundred and twenty participants presenting with varying degrees of gastroduodenal pathology from two major hospitals in Lagos were involved in the study, Lagos University Teaching Hospital (LUTH) and Lagos State University Teaching Hospitals (LASUTH). Patients presenting with symptoms relating to peptic ulcer disease such as (heartburns, acute pepperish pain, stomach pain and hotness, constipation) and or gastritis attending gastroenterology clinic and are ready to participate in the study were recruited.

Collection of Samples

All patients were properly consented and signed an informed consent form containing a full explanation of the work. Where the patient could not read, this was interpreted, in addition ethical approval

was obtained for the study. The patients underwent day case gastroscopy using an Olympus® GIF - Q30 fully immersible gastroscope under diazepam sedation with the help of gastroenterologists after due consent. Before endoscopy, blood and stool samples of the patients were taken for detection of *H. pylori* IgG antibodies, *Helicobacter pylori* stool antigen (HpSA) detection test and culture respectively.

Diagnosis

Biopsy Specimens

Three gastric mucosal antral biopsy specimens were collected from each of the dyspeptic patients that underwent gastroscopy within the study period. The first sample of the biopsy specimens was used for culture, the second for CLO test (*Campylobacter*-Like Organism test) and the third for Gram stain, while the remaining biopsy from culture was used for performing the PCR work.

Blood specimen

Blood samples were taken from the patients prior to endoscopy. This was done with the aid of sterile needle and syringe and the blood was allowed to clot, to extract the serum. Sera were used for the serological test.

Stool specimen

A sterile wide open screw-capped-spoon-attached cover plastic universal containers was provided for the participants to collect their stool sample. Fresh stools were used for the detection of *H.pylori* antigen and isolation.

Urease (CLO test)

This was performed using the CLO test kit. This is a commercially available "*Campylobacte*r – like organism (CLO) test kit from Delta West, Australia, designed to test the rapid urease production ability of *H. pylori.*

It is a semi-solid urease medium enclosed in a well and securely sealed. The medium was inoculated directly with the stomach biopsy sample by carefully immersing the biopsy in the middle of the semi – solid medium with the aid sterile needle and sealing up. Before use, the kit is usually warmed up to body temperature and after inoculation, kept close to the body (e.g in a laboratory coat pocket) until the result is read at exactly 15 minutes. A positive result is shown by a pink coloration of the medium from the original yellow color. (Figure 1)

Figure 1: Picture of the positive and negative CLO (urease) test using commercially prepared CLO test Kit (Delta West Ltd, Bentley, Australia)

KEY: A: Positive CLO test (Pink coloration of the urease medium); B: Negative CLO test (Retention of the initial yellow color of the urease medium); C: The back of the CLO test kit used for appropriate sample labeling

Polymerase Chain Reaction (PCR):

DNA Extraction

The biopsy sample were ground for 2 – 3 sec with an electric tissue homogenizer (Ultraturax; LaboModerne, Paris, France) and centrifuged for 5 mins at 10,000g to release the bacteria from the tissue. The pellet was resuspended in 300 µl extraction buffer (20mm Tris/HCl, pH 8.0,0.5% Tween 20) and proteinase K was added at a final concentration of 0.5mg/ml to destroy any proteins present. The mixture was incubated at $56^{O}C$ for one hour. Finally the enzyme was inactivated by boiling for ten minutes[19]. Five microliter of DNA was used as template for each PCR . Each sample was examined by three different PCRs. Primers used were from *vac* A (259bp) , *glm* M (294bp) and *ure* I (600bp).

PCR amplification

The following thermocycling parameters were used; $93^{O}C$ for 2 mins, and 35 cycles of $93^{O}C$ for 10 sec; $49^{O}C$ for 10 sec; $72^{O}C$ for 1.5 mins. And 1 cycle of $72^{O}C$ for 10mins, $8^{O}C$ (hold) 10mins. The amplification was done in a 50 µl reaction volume, with 5 µl of DNA used as the template for each PCR, in Gene Amp 9700 (Perkin Elmer) Machine.

Visualization of reaction products

Reaction products were visualized by running 10 µl of the reaction mixture on a 1.2% agarose gel, stained with ethidium bromide.

There were two controls, water and *H. pylori* isolate.

A biopsy is adjudged positive for *H. pylori* if 259bp DNA fragment from *H. pylori* chromosomal DNA, 600bp DNA fragment and 294bp DNA fragment were amplified with *vac* A, *Ure* I and *glm* M primers.

Serology

For the detection of *H. pylori* 1gG antibodies, an indirect solid-phase enzyme immunoassay (EIA) test kit (ImmunoComb II, orgenics, Israel) was used.

A sample was considered positive if a spot had an intensity equal to or greater than that of the positive control (anti – *H. pylori* IgG), indicating the presence of IgG antibodies to *H. pylori* (> 20 units/ml). Negative results were indicated by a spot with intensity less than that of the positive control, and an upper spot on the negative control. The kit used was equipped with a CombScan^{Tm} reflectometer, which enabled rapid and objective measurement (as relative absorbance) of the colour intensity of spots.

Gram reaction

This was done using the method of Preston and Morell[20]. A positive result is indicated by the presence of Gram negative spiral shaped organism under the cells lining the stomach mucosal wall, visualization being aided by the teasing procedure employed prior to staining, under the light microscope.

Media for isolation

Primary isolation of the organism was performed on Modified Belo Horizonte and Dent's medium [21,22]. The media consist of brain heart infusion agar base

supplemented with 10% sheep blood and Dent's supplement (10mg of vancomycin, 5mg of trimethoprim, 5mg of Cefsulodin and 5mg of Amphotericin B per liter) Oxoid, SR 147E with 40mg of Nitro-Blue Tetrazolium salt per liter to aid easier identification of *H. pylori* on media[21]. The stools were emulsified in phosphate buffered saline with the addition of 1gram of cholestyramine to dissolve the bile in the stool[23,24] prior to culture.

Helicobacter pylori Stool Antigen test (HpSA)

This was performed according to manufacturer's instruction. In brief, about 1g of stool sample was emulsified in 100μl of Phosphate Buffered Saline (PBS) and thereafter the HpSA strip was inserted into the solution. The solution moved through the strip by a process of diffusion and this was observed for 15minutes. The appearance of two distinct bands of control and test indicates a positive result, while a negative result shows only one line of control.

Stool Culture

The stool sample was emulsified in phosphate buffered saline (PBS) and pre-treated with 1g cholestyramine to remove the bile that might inhibit *H.pylori*. This suspension was then cultured on prepared culture media as described above. The resulting isolated organism was identified using rapid urease production (using Homemade urease test -HUT)[25] oxidase, catalase; hippurate hydrolysis and Gram's stain reaction.

Biopsy Culture

The biopsies were teased with sterile needle, thereafter primary isolation of the organism was performed on Dent's medium[22] as described above.

Isolation Procedure

The inoculated plates were incubated in 100% humidity at 37^{O}C for up 12 days (precisely 3 – 12 days) in microaerophilic condition specifically in carbon dioxide extinction candle jar and gas pak, before discarding if there is no growth.

The agar plates were checked for growth from day 3 through day 12. An isolate was identified as *H. pylori* on the basis of positive catalase, oxidase and urease reaction. Typical colonial morphology of small, round and greyish colonies were diagnostic and the presence of characteristic curved Gram negative short rods on Gram Stain. Other biochemical test such as Hippurate test was also used to identify the organism, *H. pylori* is Hippurate negative.

Results

Demographic characteristics and assay results for *H. pylori* infection using different methods:

Three hundred and twenty dyspeptic patients (166 males, 154 females, mean age 38.5 years (+10.8 SD, age range 10 – 85 years) were included in the study.

Of the 320 patients examined, 198 (61.8%) were *H. pylori* positive by direct Gram's stain and urease (CLO) positivity was found in 134 (41.8%). A total of 272 (85%) were seropositive for *H. pylori* 1gG, while *H.*

Table 1: Demographic characteristics and assay results for *H. pylori* infection using different methods

Age range (yrs)	Male	Female	Gram's stain	CLO test	Culture		Serology	PCR	HpSA
					Biopsy	Stool			
10-19	8	6	4Hp seen	1	3	2	16	11	14
20-29	13	17	11Hp seen	13	6	6	31	14	27
30-39	62	61	77Hp seen	81	39	22	110	70	97
40-49	43	38	59Hp seen	18	11	5	71	40	61
50-59	13	14	28Hp seen	15	3	3	28	4	24
60-69	17	13	12Hp seen	5	2	1	10	7	6
≥ 70	10	5	7Hp seen	1	3	1	6	16	5
Total	**166**	**154**	**198 (61.8%)**	**134 (41.8%)**	**67 (20.9%)**	**40 (12.5%)**	**272 (85.0%)**	**162 (50.6%)**	**234 (73.1%)**

Hp: *H. pylori*

pylori was isolated by culture from biopsies of 67 (20.9%) and stools of 40 (12.5%) patients, predominantly from patients in the age range of 30-39 years. *Helicobacter pylori* antigen was also detected in 234 (73.1%) patients and Polymerase Chain Reaction (PCR) detected *H. pylori* positivity in 162 (50.6%) of the participants (Table 1) (Figure 2).

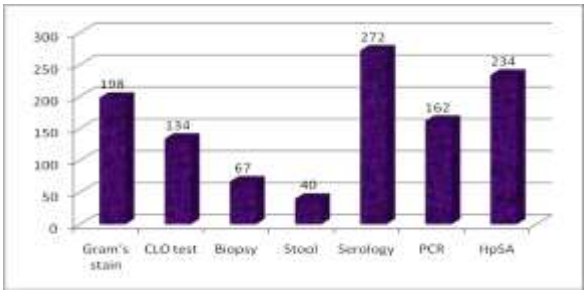

Fig. 2: Pictogram of the assay results using the different diagnostic tests.

Number of ulcer/gastritis cases positive for *H. Pylori* using the six different methods of screening/ isolation

The results from the six different methods showed that stool antigen test (HpSA) showed a higher rate of positivity (73.1%) after serology (85.0). Only 12.5% were positive through stool culture, while 20.9% were positive using the biopsies. PCR showed 50.6% positivity compared to Gram stain where 61.8% were positive. (Table 2). The differences in the results was tested statistically and found to be significant (Confidence interval (CI) of 97.5-100.0) (Figure 3).

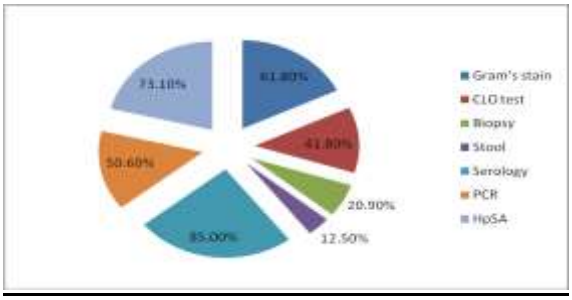

Fig.3: Percentage of *H. pylori* detected using the different diagnostic tests.

Detection of *H. pylori* by culture compared with other diagnostic methods (serology, gram reaction PCR, urease test and HPSA).

The results from this study showed 100% sensitivity using serology and HpSA. Using stool culture, the positive predictive value and specificity was also 100%. Serology showed the highest negative predictive value (100%) while stool has the highest false negative rate of 60.9%. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for culture, compared with the other five different methods are shown on Table 3.

Table 2: Number of ulcer/gastritis cases positive for *H.pylori* using the six different methods of screening/ isolation

Screening/isolation method	Number positive for *H.pylori* (%)	Number negative for *H.pylori* (%)	Total n=320
Culture (Biopsy)	67 (20.9%)	253(79.1%)	320(100.0%)
Serology	272 (85.0%)	48(15.0%)	320(100.0%)
Gram stain	198 (61.8%)	122(38.2%)	320(100.0%)
PCR	162 (50.6%)	158(49.4%)	320(100.0%)
Urease (CLO test)	134 (41.8%)	186(58.1%)	320(100.0%)
Stool (Culture)	40 (12.5%)	280(87.5%)	320(100.0%)
HpSA	234 (73.1%)	86(26.9%)	320(100.0%)

Table 3: Detection of *H. pylori* by culture compared with other diagnostic methods (Serology, Gram reaction PCR, Urease test and HpSA)

	Serology	Gram Stain	PCR	Urease (CLO test)	Stool	HpSA
Sensitivity	100.0% (97.5-100.0)	93.5% (88.7-96.5)	86.0% (80.0-90.5)	57.0% (49.5-64.2)	71.3% (61.0-80.1)	100.0% (94.6-100.0)
Specificity	22.8% (18.6-27.7)	55.8% (50.4-61.1)	75.4% (70.5-79.8)	65.9% (60.6-70.8)	100.0% (98.5-100.0)	69.2% (55.4-64.9)
PPV	44.1% (36.5-45.8)	53.2% (47.6-56.7)	65.3% (58.9-71.2)	47.3% (40.7-54.1)	100.0% (94.6-100.0)	68.6% (62.9-74.9)
NPV	100.0% (94.2-100.0)	94.1% (89.8-96.8)	90.9% (86.9-93.9)	74.0% (68.7-78.8)	90.4% (86.3-93.5)	100.0% (98.5-100.0)
FPR	58.9%	46.8%	34.7%	52.7%	0%	1.6%
FNR	0%	3.7%	10.6%	35.7%	60.9%	5.6%

PPV: Positive Predictive value; NPV: Negative Predictive value; FPR: False Positive rate; FNR: False Negative rate

Distribution of positive diagnostic tests among the studied participants

In this study according to the predefined diagnosis of infection which includes any two or more positive tests, with culture standing out as Gold standard 280 (87.5%) patients were *H. pylori* positive and 40 (12.5%) patients were *H. pylori* negative. Majority (16.3%) of the patients had stool culture, HpSA, and serology positive. Forty–one (12.8%) had HpSA and stool culture positive. In five (1.5%) patients only stool and biopsy culture were positive, they were therefore considered *H. pylori* positive, while 10 (3.1%) had only serology positive and were considered *H. pylori* negative. No test was positive in 9 (2.8%) patients (Table 4).

Table 4: Distribution of positive diagnostic tests in all samples (N=320)

Positive test(s)	Number of Samples	(%)
No positive test	9 of all samples	2.8
Serology alone	10 sera	3.1
HpSA alone	16 stools	5.0
Stool Culture alone	2 stools	0.6
Biopsy culture alone	3 biopsies	0.9
Serology and biopsy Culture	7 sera and biopsies	2.2
Serology and HpSA	38 sera and stools	11.9
HpSA and stool culture	40 stools	12.5
Gram reaction and urease	10 biopsies	3.1
Serology and PCR	4 sera and biopsies	1.3
HpSA and PCR	16 stools and biopsies	5.0
Stool culture, HpSA and serology	52 stools and sera	16.3
Gram reaction, urease and serology	16 sera and biopsies	5.0
Gram reaction, serology and PCR	30 biopsies and sera	9.4
Gram reaction, HpSA, serology and PCR	31 of all samples	9.6
Biopsy culture, serology, Gram reaction, PCR and urease	12 biopsies	3.8
Stool culture, Biopsy culture, serology, Gram reaction, PCR, HpSA and urease	23 of all samples	7.2

Discussions

This study shows that no single test alone can be regarded as the best diagnostic method for detecting *H. pylori* infection in dyspeptic patients in this environment. This is because except for serology and HpSA, which was able to detect *H. pylori* in only 3.1% and 5.0% of the patients and culture in 1.5% (stool culture and biopsy culture) of the patients, no other test was positive alone in the detection of *H. pylori* among the participants.

From the study, the best detection methods could be stool culture, HpSA and serology which detected *H. pylori* in 52 (16.3%) of the 320 patients. That means a patient can be defined as positive for *H. pylori*, even if the biopsy culture was negative, but stool culture, HpSA and serology tests were positive.

Stool culture had a sensitivity of 71.3% and specificity of 100% (when compared with other techniques). Other studies have shown culture to have a lower sensitivity for detecting *H. pylori* infection[26,27]. Many cases considered negative using urease test (CLO test) were seen to be positive using Gram stain and serology. This could be due to certain limitations to the urease test method; such as: a) the presence of some isogenic urease negative mutants reported by Dunn *et al.,*[28]; b) the fact that *H. pylori* is not the only urease positive organism that could be encountered in the oropharygeal tract, thus suggesting that some CLO positive results might have not been caused by *H. pylori.* But the Gram stain is however not limited by these factors, so is the serology test.

With a sensitivity of 57.0% and specificity of 65.9%, urease (CLO) test cannot be used for detecting *H. pylori* infection alone, although it has the advantage of having the ability to yield a positive result within 24 hours. This is in contrast to some earlier studies which tend to give a satisfactory sensitivity and 100% specificity [6,7,12] to the urease test.

Gram reaction of the biopsy is one of the methods adjudged by this study to be suitable to diagnose *H. pylori* positivity in dyspeptic patients. The method combines a lot of advantages among which includes the fact that the ingestion of anaesthetics and loss of viability of the organism during transportation does not have any effect on Gram stain detection of *H. pylori,* which grossly affected the recovery of the organism through culture[29]. Apart from all these factors, the Gram reaction is simple to perform, fast and cheap compared to other methods of detecting *H. pylori* infections in patients. The method does not involve the use of any complicated histology equipment such as the microtome (histological staining process) or PCR which requires a capital intensive PCR machine (thermocycler) or the serological method that makes use of expensive kits.

Elsewhere, endoscopic tests have been adjudged the best for the primary diagnosis of *H. pylori* infection because they allow for assessment of treatment indications[30,31]. However, culture is not recommended for routine evaluation, but it is important in populations with high prevalence of

treatment failure, especially when antibiotic resistance patterns of the organisms have to be assessed[4]. Nevertheless, the biopsy–based tests may suffer from sampling error because of the patchy nature of *H. pylori* infection, but this is of minor clinical importance[7]. The best gastric site for obtaining a positive test is the gastric angle[32,33], whereas most gastroenterologists are not that specific in obtaining biopsies. In patients who recently used therapeutic doses of antibiotics, bismuth salts and proton pump inhibitors, endoscopy should be deferred for one to four weeks to allow return of bacterial density to detectable levels[4].

Serology has been used for initial pre-endoscopy or pre-treatment screening in dyspeptic patients[31,34,35]. Nevertheless , it is of utmost importance that the performance of serological kits be validated locally[35].

In the present study, 85% of patient were seropositive by EIA, the majority (75%) having 1gG antibody titres > 160 units / ml. Endoscopic study have shown that this antibody prevalence is due to active *H. pylori* infection and is almost always associated with antral gastritis in both dyspeptic patients and asymptomatic controls[36].An antibody prevalence rate of 39% in an area of Western Nigeria, among apparently healthy individuals was reported by Olusanya [37]. This rate is relatively low compared with that found in another study by Holcombe *et al.,* 1994 who reported 80% prevalence in Northern Nigeria. The latter result is in consonance with the findings in this study. It is however, suggested that there could be significant false positivity of the serological test, because of its inability to detect true *H. pylori* infection, being hampered by cross-reaction [37]. Another possibility is that 1gG antibody titre to *H. pylori* could be high among Nigerians, as a result of past exposure. However, the latter would appear more likely and is supported by the work of Holcombe *et al*, [38] on a Nigerian population, and by Us and Hascelik,[39]who used EIA to study *H. pylori* seroprevalence in Turkey, both in a healthy population and patients with acute gastritis and duodenal ulcer[40,41]. The usefulness of serology as a tool for pre – and post-treatment diagnostic and epidemiological investigation has been evaluated[42], and 95% specificity was found in trained hands. In this study when serology was compared with culture, Gram's stain, urease and PCR using the Mc Nemar's test, a statistically significant difference ($P < 0.0001$) was observed.

Polymerase Chain Reaction has revolutionized molecular biology research and is currently broadening considerably the field of microbial diagnosis, including diagnosis of *H. pylori* infection. Nevertheless, there is still a potential for increasing its sensitivity[43]. Several studies comparing PCR detection (amplified products being detected by gel electrophoresis) with other diagnostic methods have been performed[44,45,46]. The results of these studies show that PCR can compete as an alternative diagnostic technique with culture, which is considered

today as the gold standard. From this study it is as sensitive as culture for primary detection of *H. pylori* and supposedly can give better results at treatment follow-up[47], when the number of bacteria in the gastric mucosa is usually small and the organisms may go undetected by other diagnostic methods.

The sensitivity of 86.0% and specificity of 75.4% obtained with PCR in this study have shown that PCR has the potential to be used for detection of *H. pylori* in our environment, as it far surpasses the culture and urease tests techniques. This has been confirmed by Wisniewska *et.al.,*[44]and Rimbara *et.al.*[46]. Polymerase Chain Reaction also provide a quick result[48], avoid the need for specific temperature conditions for transport of specimen, and in case of shortage in power supply during the process of amplification, all the experiment is not lost, because it can be repeated. It has also been found useful for correctly identifying infections caused by *H. pylori* [19,46]and can be easily adapted in our Laboratory for diagnostic and other purposes where constant power supply is elusive. The limitations being the fact that the equipment could be capital intensive to set up and it can only detect the DNA of the bacteria not withstanding whether the bacterial cell is viable or living or non-viable.

The current trend employed in the detection of *H. pylori* infection is to move from an invasive diagnostic methods to a non – invasive method. Though, the methods that have been earlier employed used endoscopy or gastroscopy where biopsies sample are collected from patients. However, a reliable noninvasive stool test for detection of *H. pylori* could have a large impact on the handling of patients with epigastric pain. In our study, detection of *H. pylori* by the stool antigen test (HpSA) revealed a level of sensitivity similar to that seen with the serology and a higher level of specificity (69.2%) when compared with other tests except PCR which is biopsy based (invasive test). The use of at least two out of three diagnostics tests as a gold standard for the diagnosis of *H. pylori* had been proposed earlier by others[7], therefore a combination with tests with higher sensitivity and specificity is desirable for diagnosis of *H. pylori.*

In contrast to endoscopy (biopsy based tests), stool tests have the advantage that they do not require the patient to fast before coming to the hospital. It is also easier to produce stool samples than biopsy. For follow-up screening, patients can send their stool samples directly to the microbiology laboratory; thus, less absence from work is necessary. In addition, there is a lower financial burden for the patient or the public health system (in Nigeria, the cost of endoscopy is about ₦35,000 versus ₦5,000 for the stool antigen test and or culture).

The prevalence rate of 20.9% and 12.5% (by biopsy and stool culture respectively) of *H. pylori* infection obtained in this study showed that *H. pylori* is a contributing factor to gastritis and ulcer in this part of

the world. Therefore, from this study and some others before now, we now know that many ulcers result from a bacterial infection, which are readily curable by treatment with antibiotics. Thus indicating that prevention of the spread of the organism through improved hygiene and antibiotic treatment of the organism during infection, will go a long way in reducing the burden of gastritis and peptic ulcer in this environment.

Pertaining to early diagnosis of the infection, considering invasive methods, Gram stain reaction seems to be the best method. It is least expensive and faster than the other diagnostic procedures. For good epidemiological survey, serology is the best method of diagnosis, but may be considered invasive. The use of PCR methods may also be considered. It combines the advantage of being fast with reduction of false negative results. However, considering the HpSA assay and stool culture from the non invasive methods, they seem to combine a lot of advantages that can help reduce the hurdle associated with gastroscopy such as economic loss of time, price and inconvenience coupled with the price of the fragile endoscopes. Hence HpSA, stool culture and serology could be adjudged the best diagnostic method based on this premise.

However, culture, which is the most definitive method of diagnosis, has low sensitivity. Other methods therefore have to be combined with it to facilitate the detection of *H. pylori* infection.

Before a patient can be considered positive for *H. pylori* infection, these three test; HpSA, stool culture and serology must be positive. Although, all the tests mentioned have their limitations, their various sensitivities and specificities will give a reliable result and diagnostic value in detecting *H. pylori* infection.

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