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**USE OF STOOL CULTURE AS A NON INVASIVE METHOD FOR THE DIAGNOSIS OF
HELICOBACTER PYLORI FROM STOOL OF DIARRHOEIC CHILDREN IN WESTERN NIGERIA**Oyedeki¹, K. S., Odeniyi², O. M., Ola-Bello², O.I., Ushie², S., Mustapha³, A.A. Ogunbase³, O. A.

1. Department of Medical Laboratory Science, College of Medicine, University of Lagos, Nigeria; 2. Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, Nigeria; 3. Department of Paediatrics, Mother and Child Centre, General Hospital, Gbaja- Surulere, Lagos, Nigeria.

Corresponding author: K.S .Oyedeki; Tel: +2348066117865; kolaremi903@gmail.com

ABSTRACT

Background: *Helicobacter pylori* has been associated with chronic diarrhoea, iron deficiency anaemia, growth retardation, gastric malignancies, peptic ulcer disease, and gastritis among children. Diagnosis of this infection has been invasive using biopsies while stool culture is not common or routinely practiced. This study was designed to detect and isolate *H.pylori* from stool of diarrhoeic children and highlight possible use of such for routine laboratory diagnosis of *H.pylori* infections.

Material and methods: Two hundred and sixty faecal samples obtained from diarrhoeic children were screened for *H. pylori* antigen, using *H. pylori* stool antigen test kit (HpSA) and cultured on modified DENTS medium. Cholestyramine and nitrobluetetrazolium salt were added to the stool and the medium respectively to aid isolation of *H. pylori*. Correlation of BMI and *H. pylori* infection of the children was also evaluated.

Results: Twenty-six (10%) samples showed growth on culture while 91 (35%) tested positive for *H. pylori* antigen. Of the 26 children with positive culture, 16 had a low BMI. HpSA has sensitivity and specificity of 11.5% and 62.4%. There was a significant association ($\chi^2 = 12.86$, $df=2$, $P\text{-value} = 0.004$) between age group of participants and use of HpSA kit.

Conclusion: Stool culture for recovery of *H. pylori* is feasible in our environment and diarrhoeic children should be screened for *H. pylori* using both HpSA and culture. *H. pylori* is suggested to be screened routinely especially among children having diarrhoea and are underweight. Albeit, other causes should be eliminated before concluding on the reason for the underweight.

Keywords: *Helicobacter pylori*, stool culture, HpSA, BMI, Cholestyramine, Nitrobluetetrazolium salt

**L'UTILISATION DE CULTURE DES SELLES COMME UNE MÉTHODE NON INVASIVE POUR LE
DIAGNOSTIC DE L'HELICOBACTER PYLORI DANS LES SELLES D'ENFANTS DIARRHÉIQUES DANS
L'OUEST DU NIGÉRIA**Oyedeki¹, K. S., Odeniyi², O. M., Ola-Bello², O.I., Ushie², S., Mustapha³, A.A. Ogunbase³, O. A .1. Ministère de la science de laboratoire médical, Collège de médecine, Université de Lagos, Nigeria;
2. Département de microbiologie médicale et de parasitologie, Faculté de médecine de l'Université de Lagos, Nigeria; 3. Département de pédiatrie, Centre de la mère et de l'enfant, Hôpital Général, Gbaja- Surulere, Lagos, Nigeria.Correspondence: K.S .Oyedeki ; Tel : 2348066117865 ; kolaremi903@gmail.com

RÉSUMÉ

Contexte : *Helicobacter pylori* a été associée à la diarrhée chronique, l'anémie, un retard de croissance, de cancers gastriques, ulcère gastro-duodéal, gastrite et chez les enfants. Le diagnostic de cette infection ont été à l'aide de biopsies invasives tout en culture des selles n'est pas commune ou pratique courante. Cette étude a été conçu pour détecter et isoler *H. pylori* dans les selles d'enfants diarrhéiques et mettre en évidence l'utilisation possible d'une telle routine de diagnostic en laboratoire des infections à *H. pylori*.

Matériel et méthodes : Deux cent soixante échantillons de selles diarrhéiques obtenues à partir d'enfants ont été examinés pour l'antigène d'*H. pylori*, à l'aide de *H. pylori* antigènes selles test kit (HpSA) et cultivées sur milieu modifié des bosse

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La cholestyramine et nitrobluetetrazolium ont été ajoutés à la selle et le support de l'isolement à l'aide respectivement de *H. pylori*. Corrélation entre l'IMC et l'infection à *H. pylori* des enfants a également été évaluée.

Résultats : Vingt-six (10 %) échantillons montrent une croissance de la culture tandis que 91 (35 %) ont été testés positifs pour *H. pylori* antigène. Des 26 enfants avec culture positive, 16 avaient un faible IMC. HpSA a la sensibilité et la spécificité de 11,5 % et 62,4 %. Il y avait une association significative ($\chi^2 = 12,86$, $df = 2$, $P\text{-value} = 0,004$) entre le groupe d'âge des participants et l'utilisation de kit HpSA.

Conclusion : culture des selles pour récupération de *H. pylori* est possible dans notre environnement et les enfants diarrhéiques devraient être examinés pour *H. pylori* en utilisant à la fois les HpSA et de la culture. *H. pylori* est suggéré pour être contrôlés régulièrement en particulier chez les enfants ayant la diarrhée et une insuffisance pondérale. Bien que d'autres co-fondateurs doivent être éliminés avant de conclure sur la raison de l'insuffisance pondérale.

Mots clés: *Helicobacter pylori*, culture des selles, HpSA, BMI, la cholestyramine, sel de Nitrobluetetrazolium

INTRODUCTION

Helicobacter pylori is a motile, microaerophilic Gram negative curved rod bacterium that inhabits the gastric mucosa of human stomach. and has been recognized as class 1 carcinogen [1]. The demonstration of its involvement in gastro duodenal pathologies has basically changed the perception of people about the disease [2]. Infection with the organism causes peptic ulcers, gastritis, duodenitis and gastric cancers [3,4,5]. It has been reported that more than half of the world's population are infected with the organism in both developed and developing countries [5]. Factors that influence the acquisition of *H. pylori* in childhood is basically overcrowding and the socio-economic condition of the parents [6-8]. The mode of transmission of childhood infection has been found to be common among parents who pre-masticate food for their children [9]. Most of the available evidence supports person-to-person transmission by faecal-oral, oral-to-oral and gastric-to-oral routes [9-12]. In children, gastric inflammation could cause low gastric secretion resulting in impaired "gastric barrier" associated with increased susceptibility to enteric infections, which is a major public health concern linked to diarrhoea, malnutrition and growth retardation in developing countries [13,14]. *H. pylori* infection can be transmitted orally through faecal matter originating from ingestion of waste-tainted food or water [15]. It is also possible that *H. pylori* could be transmitted from the stomach to the mouth through belching or gastro-oesophageal reflux, with common symptoms of gastritis, when small amount of the stomach's contents is involuntarily forced up to the oesophagus. The bacterium could then be transmitted through oral contact [16]. More than half of the world's population is infected with *H. pylori*, which is acquired almost always within the first 5 years of life [17]. The possible routes are faeco-oral, oral-oral and gastro-oral [16,18]. Thomas and associates, were able to isolate *H. pylori* DNA and also culture *H. pylori* from human faeces thereby, suggesting faeco-oral transmission [19]. Oral transmission has also been proven because

H. pylori was isolated from dental plaques and saliva [20].

The prevalence of the disease varies depending on the method of diagnosis. Using serology 15% and 46% prevalence rates were recorded among Gambian children aged less than 20 months and 40-60 months respectively [21] and 45% among Indian children [22]. In Bolivia and Alaska, the seroprevalence were 70% and 69% among the 9 years old respectively [22]. While, the seroprevalence in preschoolers in Brazil was found to be 69.7% [23].

In developing countries, studies have suggested that, *H. pylori* may be associated with chronic diarrhoea especially among children [21] and that *H. pylori* at a young age may induce hypochlorhydria which interferes with the normal acidic barrier in the stomach. However more works need to be done among children in order to determine whether eradication of *H. pylori* reduces the prevalence of chronic diarrhoea [21]. Low socioeconomic status and overcrowding are some of the predisposing factors to the infection [24].

Diagnosis of *H. pylori* are divided into invasive and noninvasive techniques [25,26]. Invasive tests need an Upper Gastrointestinal Endoscopy (UGIE), while the noninvasive techniques do not require endoscopy, but involve methods that make use of stool using kits such as HpSA, stool culture and Urea Breath Tests (UBT) [27]. Generally, biopsy cannot be justified, especially in children, unless one wishes to isolate the organism for antibiotic sensitivity testing or there is a clear clinical indication for UGIE [28]. Moreover, if one opted to test for *H. pylori* by biopsies with UGIE, it requires specimen from multiple regions of the stomach, and this may be too stressful for the child. However, a noninvasive and diagnostic tool for detecting *H. pylori* infection is more desirable in pediatrics, because the upper gastrointestinal endoscopies in young children are usually performed in intubation anesthesia or conscious sedation [29] and paediatric endoscopy is very costly and not common [28,30,31]. Other non-invasive methods such as Urea Breath Test (UBT) are expensive, difficult to administer in young children and not available in all

countries though it is as reliable as the invasive methods [13]. Despite the fact that recovery of *H. pylori* from stool culture is laborious, difficult, hence not usually practiced [32] coupled with the fact that culturing of this fastidious organism is relatively difficult, expensive and needs special media for growth; culture still remains the Gold standard [33]. The culture of *H. pylori* has two major advantages: Firstly, it allows antimicrobial susceptibility testing and secondly, the isolates that are obtained by culture can be further studied for its characteristics [25, 34].

During culture of *H. pylori*, patients are advised to stay off antibiotics because it is often more difficult to isolate the organism [35]. The addition of Nitroblue tetrazolium (NBT) salts to Columbia agar base (Oxoid) and horse serum (Oxoid), aid in the identification of *H. pylori* colonies cultured on agar media [25,35] while the addition of cholestyramine to the stool help in dissolving the bile in the stool to aid the isolation of *H.pylori* if present[36].

In Nigeria, the first work done on *H.pylori* was by Coker and Akande [37] and it employed invasive method using biopsy. However, the current trend employed in detection and isolation of *H. pylori* infection is to move from an invasive diagnostic methods to non-invasive method[38]. *H. pylori* stool antigen (HpSA) test is non-invasive method which only detects the antigen in the faecal sample, and does not include the isolation of the organism[39] The limitation to the use of this method is that antibiotics susceptibility testing cannot be done without isolation of the organism. Hence culture of the organism from the faecal sample needs to be established in routine practice to enable effective management of the infection. This study was therefore designed to detect and isolate *H. pylori* from stool of diarrhoeic children in Lagos using HpSA test and culture respectively.

MATERIALS AND METHODS

Sampling Technique:

The stool samples were collected with a sterile bottle, wide open with attached screw-capped spoon plastic universal containers. A total of 260 stool samples were obtained from diarrhoeic children attending Paediatric Clinics in some health facilities in Lagos metropolis. Consent and assents from parent, guardian and children was sort for respectively depending on age. The parents helped in filling the questionnaires, detailing the weights and heights of the children to enable calculation of their Body Mass Index (BMIs), using the BMI Percentile graph. There was no criterion for hospital selection as samples were collected with only the prognosis of diarrhoea. The inclusion criteria were children within the age range of ≤ 1 to 16 years having diarrhoea and whose

parents consented and the child assented as the case may be before recruitment into the study. Children who have been on antibiotics for two weeks prior to sample collection were excluded.

Sample Processing: The stool samples were processed using detection method- *H.pylori* stool antigen (HpSA) test kit (SD^{BIO}LINE *H.pylori* Ag, Germany) and culture method on modified Dent's medium. The stool antigen assay was carried out according to the manufacturer's instructions. Three mls of the assay diluents was transferred into the desired collection tube for use and one gram of faecal sample was emulsified into it using sample collection swab stick provided. The swab was swirled for at least 10 times. This collection swab was discarded after squeezing it against the wall of the collection tube. The resulting suspension was allowed to stand for 5 minutes., then three drops of the suspended supernatant was added into the sample well(s) of the cassette test device and the assay was left to run for fifteen minutes. Appearance of two test lines i.e. "C" and "T" which are the Control and Test lines in the result window, indicates a POSITIVE RESULT. While one test line "C", NEGATIVE RESULT.

Stool culture

The stool sample was emulsified in phosphate buffered saline and 1gram of cholestyramine was added to the suspension[40]. This is to dissolve the bile in the stool. The emulsion was filtered using sterile muslin cloth to remove stool debris. Filtrate further filtered using membrane filter of pore size 0.45 μm as it is expected to retain *H. pylori* if present in the stool. The membrane filter was now cultured for a period of 3 to 12days in a microaerophilic atmosphere (5% O₂, 10%CO₂ and 85% N₂) using the anaerogen gas pak (Oxoid-England) at 37°C on modified Dent's medium (Oxoid, England) [41,42] into which Nitroblue tetrazolium salt (NBT) was added[43], the latter was supposed to aid the appearance of *H. pylori* on the culture plate, if there is any growth [41]. Plates were checked intermittently for sub culture after the first 3 days through to the 12th day before discarded as no growth. Colonies appearing very tiny, pin head size, with a shining grey features were sub cultured for further testing to characterize *H. pylori*. The isolates were Gram negative spiral rods and produce urease, oxidase, and catalase enzymes during preliminary characteristic reactions.

Antibiotics Susceptibility

The antibiotic susceptibility testing was done using the modified Kirby Bauer [44] technique using the disk diffusion method. Two to three colonies of the

Testing:

H. pylori isolates were emulsified with physiological saline dispensed into sterile universal container. This suspension was standardised to 0.5 MacFarland standard. Isosensitest agar (Oxoid- England) was employed for this test with the addition of 10% laked horse blood (Oxoid-England). This has been reported to be suitable for carrying out antibiotic susceptibility testing on *Helicobacter pylori* isolates [45]. The antibiotics used were; Metronidazole, Ciprofloxacin, Clarithromycin, Amoxicillin, Erythromycin, Gentamicin with a herbal extract usually used for peptic ulcer. The seeded plates were incubated under microaerophilic condition overnight at 37°C [44,49].

Calculation of the Body Mass Index

The Body Mass Index of the participants were calculated by taking the weights (kg) against heights in squared (m²). The BMIs were extrapolated from BMI Percentile graph (Children BMI scale) for the actual body mass. These were classified into the categories of normal weight, low weight, overweight or obese depending on the BMI of the participants [46] (Figure 1).

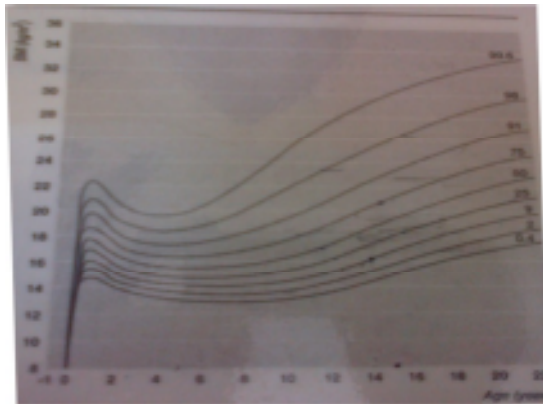


FIGURE1: BMI PERCENTILE GRAPH FOR CHILDREN
BMI PERCENTILE GUIDE (Percentile)
Underweight: Below 5; Normal weight: 5 - 85; Overweight: 86 - 96; Obese: 97- 99

Questionnaire Analysis

Information regarding the predisposing factors to *H.pylori* infection among the population studied was collected through the questionnaires administered to the parents or guardian of the participants. The questionnaires were analyzed using the Statistical Package for Social Sciences (SPSS) version 20 to get the information concerning the possible predisposing factors to *H. pylori* infection.

Ethical Considerations: The ethical approval for this study was obtained from Lagos University Teaching Hospital Health Research Ethics Committee. The consent of the participants was sought before recruitment into the study while confidentiality of all participants' information was maintained.

RESULTS

Socio-demographic characteristics of participants:

Of the 260 participants recruited for this study, 143 (55.0%) were males and 117 (45.0%) were females, with a male: female ratio 1: 1.2. The age ranges between 27 days to 16.1 years while the mean age and standard deviation (SD) are 1.45 ± 0.498 years (Table 1).

Frequency distribution of detection and isolation of *H. pylori* from stools of diarrhoeic children:

H. pylori antigen was detected in 91 (35.0%) participants using HpSA test kit while the organism was isolated from 26 (10%) of the stool samples using culture (the Gold standard). There was no statistical significant relationship ($\chi^2 = 6.98$, $df = 1$, P -value = 0.085) between the use of culture method and HpSA in diagnosing *H. pylori* infection from diarrhoea stool samples in this study (Table 2).

Comparison of performance characteristics of HpSA test kit and culture

The performance characteristics for HpSA show a sensitivity of 11.5%, specificity of 62.4% while the positive predictive value and negative predictive value were recorded as 3.3% and 86.4% respectively (Table 3).

Correlation of *H. pylori* infection with Body Mass Index (BMI) of the studied participants

Of the 26 (10%) *H. pylori* positive stool samples, 15 (57.7%) were underweight (< 5 percentile), 8 (30.8%) had a healthy weights (5 - 85 percentile) while 3 (11.5%) were obese based on the percentile of 96 - 99 (Figure 2).

Correlation of *H. pylori* antigen detection with Body Mass Index (BMI) of the studied participants

Out of the 91 (35.0%) *H.pylori* positive participants , 73 (80.2%) were underweight (< 5 percentile), 8 (8.8%) had a BMI percentile range of 5- 85, 7(7.7%) had a BMI percentile between (86 - 95) while 3(3.3%) had a BMI percentile range of (96 -99), which was an indication of obesity(Figure 3).

Prevalence of *H. pylori* infection among the studied population

From the 260 participants enrolled for this study, only 26 (10%) showed growth of *H. pylori*. after culture for 3-12days (Figure 4).

TABLE I: SOCIO-DEMOGRAPHIC CHARACTERISTICS OF PARTICIPANTS

Variables	Attributes	Respondents	
		Number	Percentage
1. Sex	Male	143	55.0
	Female	117	45.0
2. Age group	≤ 1 year	91	35.0
	2-5 years	111	42.6
	> 5 years	57	21.9
3. Number of members living together at home	3-5 persons	216	83.1
	6-9 persons	44	16.9
4. Type of apartment	One room apartment	60	23.1
	Room and parlor	60	23.1
	Flat	130	50.0
	Duplex	10	3.8
5. Source of water supply	Borehole	255	98.1
	Well	3	1.1
	Spring	3	1.1
6. BMI Percentile	< 5 percentile	156	60.0
	5-85 percentile	78	30.0
	96-99 percentile	26	10.0
7. Washing of hand after toileting	Yes	257	98.8
	No	3	1.1
8. Feeding habit (Chewing food for infant)	Yes	26	10.0
	No	234	90.0

Antibiotics susceptibility patterns of *H. pylori* isolates from the stool samples

Majority of the *H. pylori* isolates 21 (80.8%) were sensitive to Ciprofloxacin with zone of inhibition size of 35mm. Eight (30.8%) were sensitive to Gentamicin

with a zone sizes of 4mm while only 5 (19.2%) with a zone sizes of 10mm were sensitive to Erythromycin . However, *H.pylori* showed moderate sensitivity to the other antibiotics used in this study (Table 4).

TABLE 2: FREQUENCY DISTRIBUTION TABLE SHOWING ISOLATION AND DETECTION OF *H. PYLORI* AMONG THE STUDIED POPULATION

Variables	Attributes	Participants	
		Number	Percentage
N=260			
<i>H. pylori</i> stool antigen (HpSA) test	Positive	91	35.0
	Negative	169	65.0
Stool culture status	Positive (<i>H. pylori</i> -isolated)	26	10.0
	Negative (<i>H. pylori</i> not isolated)	234	90.0

TABLE 3 : COMPARISON OF PERFORMANCE CHARACTERISTICS OF HPSA RAPID TEST TECHNIQUE AND THE USE OF CULTURE METHOD (GOLD STANDARD)

H.pylori Ag. detection using HpSA	Culture of <i>H.pylori</i>		Total
	Number Positive (%) (<i>H.pylori</i> isolated)	Number Negative (%) (<i>H.pylori</i> not isolated)	
Number Positive (%) (<i>H. pylori</i> Ag Present)	3 (3.3)	88 (96.7)	91(100)
Number Negative (%) (<i>H. pylori</i> Ag absent)	23 (13.6)	146 (86.4)	169 (100)
Total	26 (10.0)	234 (90.0)	260 (100)

$\chi^2 = 6.98$, $df = 1$, $P = 0.085$; Sensitivity (%) = 11.5; Specificity (%) = 62.4; Positive Predictive Value(%) = 3.3; Negative Predictive Value(%) = 86.4

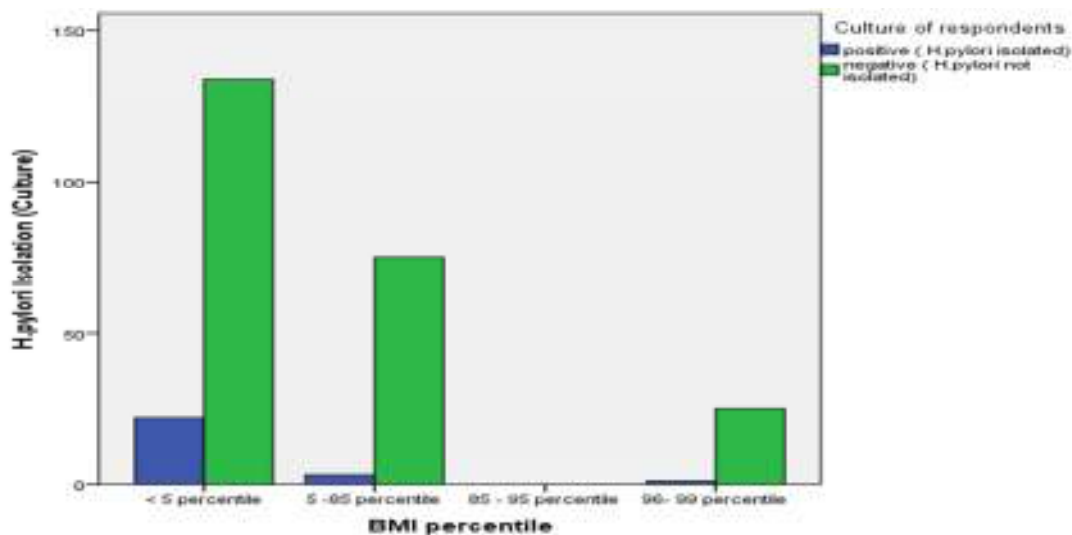


FIGURE 2: SHOWING CORRELATION OF *H. PYLORI* ISOLATION OF DIARRHOIEC CHILDREN AGAINST BMI PERCENTILE

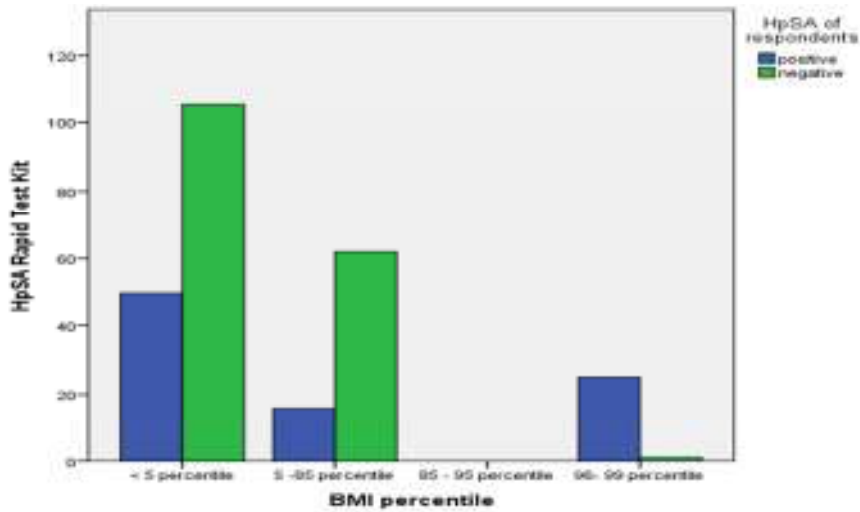


FIGURE 3: SHOWING HPSA RAPID ANTIGEN DETECTION TEST STATUS OF DIARRHOEIC CHILDREN AGAINST BMI PERCENTILE

TABLE 4: ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF THE *H. PYLORI* ISOLATES

Antibiotics	Resistance (N=26) (%)	Sensitive (N=26) (%)	Zone size Range (mm)
Amoxicillin (10 µg)	11 (42.3)	15 (57.7)	15-23
Ciprofloxacin(5µg)	5 (19.2)	21 (80.8)	34-42
Clarithromycin(15µg)	8 (30.8)	18 (69.2)	25-31
Metronidazole(50µg)	11 (42.3)	15 (57.7)	6-8
Gentamicin(10µg)	18 (69.2)	8 (30.8)	15-18
Erythromycin (15 µg)	21 (80.8)	5 (19.2)	25-30
Herbal extract A(20µg)	26 (100)	0 (0)	6-8)
Herbal extract B(20µg)	26 (100)	0(0)	6-8) Metronidazole zone range adapted

DISCUSSION

Helicobacter pylori has been found to be associated with several disease conditions among which includes gastritis, gastric ulcer, iron deficiency anaemia, gastric cancer, stunted growth, diarrhoea etc among children [13,47]. In this study *H. pylori* has been incriminated as one of the aetiological agents of diarrhoea among children in Western Nigeria with the prevalence of 10.0% using culture method. This result corroborates similar works done elsewhere [19,36,38]. Thus suggesting the implication of *H. pylori*

as gastrointestinal pathogen and the need for the routine screening of diarrhoeic stools for *Helicobacter pylori* infection rather than reporting no pathogens found especially when *Salmonella* or *Shigella* species were not isolated.

Before now there had been emphasis on invasive methods for diagnosis of *H.pylori* using biopsies. However, this study has detected and isolated *H.pylori* from stool - a non-invasive method using *H. pylori* stool antigen (HpSA) kit and culture technique. The HpSA stool antigen kit is convenient

especially when dealing with children as it does not involve surgery nor discomfort when using Urea Breath Test, serology or endoscopy. Therefore the inconveniences caused by process of endoscopy, anaesthesia, cost of paediatric endoscopes is reduced or virtually inexistent as stool samples can be used in place of biopsies for the diagnosis of *H. pylori* infection.

In this study a detection rate of 35.0% using HpSA kit was recorded and this compares with work of Smith *et al.*[27] where, a detection rate of 36.7% was recorded among dyspepsia patient undergoing upper gastrointestinal endoscopy. There was a statistical significant relationship between the age of participants and their *H. pylori* infection status as previously inferred by Tahereh *et al.*[48] where incidence of *H. pylori* was synonymous with increasing age of the studied population. This may be ascribed to increasing biomass of the organism as they multiply in their host with increasing age. Among the participants that were positive by cultural method for *H. pylori* infection, some of them were underweight and when tested statistically there was a significant association between the presence of *H. pylori* and low body mass index of these children. This result is in consonance with the result of Oderda *et al.*[13] implicating *H. pylori* in stunted growth of children in Italy.

The antibiotics susceptibility result obtained tallies with those recorded by Oyedemi *et al.* [34] and this implies that the quinolones such as ciprofloxacin still shows good activity against *H. pylori* strains. However, it recorded lower resistance to amoxicillin and metronidazole than what was previously obtained in some studies [34,49]. Though, increase in resistance to metronidazole has also been reported by Henriksen and his associates [50]. This may be explained based on age difference of the study population compared, possible differences in exposure to antibiotics of these populations and antibiotics abuse, where one antibiotic may be used for treating different ailments. Therefore, one can conclude that the *H. pylori* isolates in this study may isolation is desired for possible antibiotics susceptibility testing. It should however, be noted that, prior to the use of HpSA, researcher should alert participants, parent/guardian on the need to abstain from antibiotics, as this may give a false negative result due to the effect of the antibiotics on the organism. Evaluating the culture method, though it has low sensitivity, it is highly specific and desirable when considering antibiotics sensitivity testing. However, there are some challenges posed with this method such as incessant and erratic power outage coupled with the fact that it is cumbersome, despite

probably be from supposedly antibiotic-naive children compared to antibiotics exposed adults with subsequent drug abuse and possible self-medication which may be responsible for the discrepancy observed [34,42]. Other factors could be the fact that, the result of antibiotics sensitivity testing of *H. pylori* is determined and or dependent on the type of media, sufficient incubation timing and as well the growth condition.

Current treatment strategies to eradicate *H. pylori* in children have been developed primarily by using data from adults [51]. This is not good enough as it is scientific to always perform antibiotics susceptibility testing for different strains of organisms before determining the best regimen for the treatment of the infection caused by these organisms [57]. This is because organisms from different age groups, location in the body and from different samples may exhibit different sensitivities as is observed in this study [52,53]. A triple therapy has been considered to be the standard treatment for children; a proton pump inhibitor combined with two antibiotics has been shown to be very effective in clearing *H. pylori* from the stomach [54]. The current recommendation is treatment with amoxicillin, clarithromycin and a proton pump inhibitor for 2 weeks [55]. However, another triple therapy regimen that are effective in children has been shown to include a proton pump inhibitor combined with Clarithromycin and Metronidazole [55], or amoxicillin and metronidazole combined with Bismuth that would be given for a duration of 2 weeks [56]. This treatment regimen seems to agree with the result of the antibiotics susceptibility testing obtained in this study.

Expatriating on the comparative advantage of the non-invasive method of diagnosis used in this study, it can be suggested that HpSA is easy to perform, patient friendly as no anaesthetics is involved and it is also cheaper compared with Urea Breath Test (UBT). Though the latter may be classified as non-invasive, its cost is similar to those of endoscopy test and not as patient friendly as HpSA stool antigen kit[57]. However, the limitation of HpSA is that, it is a qualitative based test and may not be useful if this it is the most advocated method for diagnosis of *Helicobacter pylori* based on its comparative advantages over other methods.

On the possible predisposing factors to the transmission of the infection through overcrowding, there was no significant association between number of occupant and *H. pylori* infection in this study. Other possible mode of transmission as postulated by [58,59] Mohammed *et al.*, and Ramy *et al.*, [59,60] such as oral-oral and faeco-oral routes may be implicated as corroborated in this study.

CONCLUSION

This study revealed a prevalence of 10% *H. pylori* infection among children having diarrhoea disease using culture method. This suggests that *H. pylori* may be one of the incriminating pathogens in diarrhoea disease among children. It also imply that, we may need to routinely screen for *H. pylori* in the stool of children having gastrointestinal problems, especially if no other supposed pathogens such as *E. coli*, *Salmonella* and *Shigella* are found.

The diagnosis of *H. pylori* infection using stool culture is more accurate method for diagnosis of the organism, though it has low sensitivity compared with *H. pylori* stool antigen (HpSA) [57]. Despite the fact that culture is laborious, it is advocated because of its importance in the effective antibiotics management of the infection. This is based on ability

to do the *in vitro* susceptibility testing of the organism involved. Furthermore, because of the expertise required in culture procedure, incubation time especially *H. pylori* (12days) and its cumbersome nature, *H. pylori* stool antigen (HpSA) rapid test kit may be used instead in detecting the *H. pylori* antigen which means treatment of such cases will be empirical, since no isolation is involved.

Finally, this study has shown that *H.pylori* is culturable from stool in our environment; it is also involved in other gastrointestinal problems aside peptic ulcer disease and gastritis and may also be a co-factor in stunted growth in children. It is therefore imperative that samples from gastrointestinal tract relating to gastrointestinal problems may also be screened for *H.pylori* especially when children are involved.

REFERENCES

1. Kawaguchi K., Matsuo J., Osaki T., Kamiya S., Yamaguchi H. Prevalence of *Helicobacter pylori* and *Acanthamoeba* in natural environment. *Letters in Applied Microbiology*. 2009; Volume 48, Issue 4, 48: 465-471.
2. Marshall MJ., Warren RJ. Unidentified curved bacilli on gastric epithelium active chronic gastritis. *Lancet*, 1983; 1: 1273-1275.
3. Fritz LE., Slavik T., Delpont W. Incidence of *Helicobacter felis* and the effect of coinfection with *Helicobacter pylori* on the gastric mucosa in the African population. *J. Clin. Microbiol.* 2006;44(5): 1692-1696.
4. Figueroa G., Troncoso M., Toledo M. Prevalence of serum antibodies to *Helicobacter pylori* *VacA* and *CagA* and gastric diseases in Chile. *J. M. Microbiol.* 2002;51: 300-304.
5. Ahmed KS., Khan AA., Ahmed I . Impact of household hygiene and water source on the prevalence and transmission of *H. pylori*: a South Indian perspective. *Singapore Med. J* 2007;48(6): 543-549.
6. Feldman, RA. Epidemiologic observations and open questions about disease and infection caused by *Helicobacter pylori*. In Achtman M and Suerbaum S (eds) *Helicobacter pylori*: molecular and cellular biology. Wymondham, United Kingdom, Horizon Scientific press, 2001; pp. 29-51.
7. Ndip RN., Malange EA., Akoachere TKJF . *Helicobacter pylori* antigens in the faeces of asymptomatic children in the Buea and Limber health districts of Cameroon: A pilot study. *Trop. Med. Int. Health*, 2004;9(9): 1036-1040.
8. Dube C., Nkosi TC., Clarke AM. *Helicobacter pylori* in an asymptomatic population of Eastern Cape Province, South Africa: Public health implication. *Rev. Environ. Health*, 2009b; 24(3): 249-255.
9. Mladenova I., Durazzo M., Pellicano R. Transmission of *Helicobacter pylori*: are there evidences for a fecal-oral route? *Pub Med.* 2006; 97(1):15-8.
10. Hardin FJ., Wright RA. *Helicobacter pylori*: review and update. *Arch. Hosp. Physician* 2002; 38(5): 23-31.
11. Asrat D., Kassa E., Mengistu Y. Antimicrobial susceptibility pattern of *Helicobacter pylori* strains isolated from adult dyspeptic patients in Tikur Anbassa University Hospital, Addis Ababa. *Ethiop. Med. J.* 2004; 42: 79-85.
12. Dube C., Tanih NF., Ndip RN. *Helicobacter pylori* in water sources: A global environmental health concern. *Rev. Environ. Health*, 2009a; 24(1): 1-14.
13. Oderda G., Rapa A., Ronchi B. Detection of *Helicobacter pylori* in stool specimens by noninvasive antigen enzyme immunoassay in children: multicentre Italian study. *BMJ*, 2000; 320 : 347-348.
14. Thomas JE., Dale A., Bunn JE. Early *Helicobacter pylori* colonization: the association with growth faltering in The Gambia. *Arch. Dis. Child.* 2004;89(12): 1149-1154.
15. Santiago p., Moreno Y., Ferrus MA. Identification of Viable *Helicobacter pylori* in Drinking Water Supplies by Cultural and Molecular Techniques. *EPublications*. 2015; 20(4): 252 - 259.
16. Mulchandani R., Hans-Olof N., Wadström T., Joshi, BR. *Helicobacter pylori* : Is it Transmitted Through Faecal-oral or Oral-oral Route? *Journal of Pure and Applied Microbiology*. 2013; 7(3). p.2429-2438.
17. Megraud, F., Lehours, P., Vale, F.F. The history of *Helicobacter pylori*: from phylogeography to paleomicrobiology. *Journal of Clinical*

- Microbiolgy and Infection.2016; Volume 22, Issue 11, Pages 922-927.
18. Gold, BJ. *Helicobacter pylori* infection in children. *Curr Prob Pediatr*. 2001;31:247-66.
 19. Guetarni H., Bensoltane A. Isolation And Characterization Of *Helicobacter pylori* Strains From Gastric Biopsies Of Algerian Patients . *OnLine Journal of Biological Sciences*.2013; 13 (2): 41- 49.
 20. Goosen C., Theron J., Ntsala M. Evaluation of a novel heminesteds PCR assay based on the phosphoglucosamine mutase gene for detection of *Helicobacter pylori* in saliva and dental plaque. *J Clin Microbiol*. 2002; 40:205-209.
 21. Dulciene MMQ., Andreia MCR., Jean EC. Unintended consequences of *Helicobacter pylori* infection in children in developing countries . Iron deficiency, diarrhea, and growth retardation.. *Journal of Gut Microbes*. 2013; Volume 4, Issue 6Pages 494-504 .
 22. Kate V., Ananthkrishanan N., Ratnakar C. Anti-*H.pylori* IgG seroprevalence rates in asymptomatic children and adults form south India. *Indian J Med Microbiol*. 2001;19:20-25.
 23. Rocha GA., Rocha AM., Silva LD. Transmission of *Helicobacter pylori* infection in families of preschool-aged children from Minas Gerais, Brazil. *Trop Med Int Health*, 2003;8:987-991.
 24. Wizla-Derambure N., Michaud L., Ategbo S. Familial and community environmental risk factors for *Helicobacter pylori* infection in children and adolescents. *J Pediatr Gastroenterol Nutr*. 2001;33:58-63.
 25. Wang YK., Kuo FC., Liu CJ., Wu MC., Shih HY., Wang SS., Wu JY., Ku CH., Huang YK., Wu DC. Diagnosis of *H. pylori* Infection : Current Option And Developments. *World J Gastrolenterol*.. 2015; 21 (40):11221 - 11235.
 26. Smith SL, Oyedeji KS., Odeniyi OA. Diagnosis of *Helicobacter pylori* infection among patients with dental caries by stool antigen test. *Br J Biomed Sci*. 2006;63(3): 144-145.
 27. Smith SL, Omonigbehin EA., Goodluck HA . Diagnostic methods for detection of *Helicobacter pylori* in Nigeria. *Journal Tropical Gastroenterology*, 2010;31(2):113-115.
 28. Shaman R., Niranga MD., Janaka de Silva H. *Helicobacter pylori* infection in children. *Sri Lanka Journal of Child health*, 2009; 38: 86-88
 29. Braden B., Posselt H., Ahrens P . New immunoassay in stool provides an accurate noninvasive diagnostic method for *Helicobacter pylori* screening in children. *Pediatrics*; 2000; 106: 115-117.
 30. Tom, RO . Upper gastrointestinal endoscopic findings in adolescents at Lacor hospital, Uganda *Afr Health Sci*. 2006; 6(1): 39-42.
 31. Miller V., Doig C M . Upper gastrointestinal tract endoscopy Personal practice *Archives of Disease in Childhood*, 1984;59, 1100 -1102
 32. Dore MP., Osato MS., Malaty HM . Characterization of a culture method to recover *Helicobacter pylori* from faeces of patients. *Helicobacter* ,2000;5: 165- 168
 33. Takahiro U., David YG. Diagnosis of *Helicobacter pylori* using the rapid urease test *Ann Transl Med*. 2015; 3(1): 9.
 34. Oyedeji KS., Smith SL, Coker AO. Antibiotic susceptibility patterns in *Helicobacter pylori* strains from patients with upper gastrointestinal pathology in Western Nigeria. *Br. J.Biomed Sci*. 2009;66 (1): 10-3.
 35. Wang WH ., Wong WM. , Dailidiene D. Aspirin inhibits the growth of *Helicobacter pylori* and enhances its susceptibility to antimicrobial agents *Gut* 2003;52:490-495 doi:10.1136/gut.52.4.490
 36. Tahereh F., Nargess V., Mehri N. Culture of *Helicobacter pylori* from stool samples in children. *Canadian Journal of Microbiology*, 2007; 53(3): 411 - 416, 10.1139/W06-144
 37. Coker A.O and Akande, B. Isolation of *Campylobacter pyloridis* from Nigerian patients with gastroduodenal pathology. *West African Journal of Medicine* 1989; 8 (2) :106 - 110.
 38. Mohammad K ., Fereshteh S ., Hossein A ., Naser E.D., Mohammad R.P., Mostafa H., Amir G., Mohammad H.S. Comparison of five diagnostic methods for *Helicobacter pylori*. *Iran J Microbiol*. 2013; 5(4): 396 - 401.
 39. Abolfazl I., Mohammad -Reza G., Saeed S., Hosein S., Akram H., Mohadeseh M. Stool Antigen Tests for the Detection of *Helicobacter pylori* in Children. *Iran J Pediatr*. 2013; 23(2): 138 - 142.
 40. Ndip R. N., MacKay W. G., Farthing M.J. G., Weaver L. T. Culturing of *Helicobacter pylori* from clinical specimens. *Journal of Pediatric Gastroenterology and Nutrition*. 2003;_Volume 36 - Issue 5 - pp 616 - 622
 41. Bury-Moné S., Kaakoush NO Asencio C., Mégraud F., Thibonnier M., De Reuse H., Mendz GL. Is *Helicobacter pylori* a true microaerophile? 2006; 11(4):296-303.
 42. Dulciene M. M.Q., Edilberton N. M., Gilfone A. R. Indicator Medium for Isolation of *Campylobacter pylori*. *Journal of Clinical Microbiology*.1987;; p. 2378-2379.
 43. McNulty C., Robert O., David T., Peter H., Kenneth M., Ashley P., Gillian S ., Louise T., and the PHLS *Helicobacter* Working Group. *Helicobacter pylori* susceptibility testing by disc diffusion . *Journal of Antimicrobial Chemotherapy*. 2002; Volume 49, Issue 4, Pages 601-609.
 44. Michael S. , Ina T., Cosima L., Jan B., Julia M., Arne K., Mariya V., Thomas W., Dirk S., Peter M. Different antibiotic susceptibility between antrum

- and corpus of the stomach, a possible reason for treatment failure of *Helicobacter pylori* infection. *World J Gastroenterol.* 2014; 20(43):16245 - 16251.
45. Apostolopoulos P., Kousmoutsos I., Ekmektzoglou K.. Concomitant versus sequential therapy for the treatment of *Helicobacter pylori* infection: a Greek randomized prospective study *Scand J Gastroenterol.* 2016; 51(2):145- 151
 46. Mahir G., Ahmet O., Hande O K.. Impact of *Helicobacter pylori* on growth : Is The Infection or Mucosal Disease Related to Growth Impairment? *J Digestive Diseases and Sciences.* 2010;Vol. 55 Issue 10, pp 2878 -2886.
 47. Dulciene MMQ., Andreia MCR., Jean EC. Unintended consequences of *Helicobacter pylori* infection in children in developing countries. *Gut Microbes.* 2013; 4(6): 494-504.
 48. Tahereh F., Nargess V., Shayesteh S . Application of a stool antigen test to evaluate the incidence of *Helicobacter pylori* infection in children and adolescents from Tehran, Iran. *Clin. Diag. Lab. Immunol.* 2005; p 1094-1097.
 49. Clinical and Laboratory Standards Institute(January,2013). Antimicrobial susceptibility testing standards.
 50. Henriksen TH., Lerang F., Lia A., Schøyen R., Thoresen T., Berge., Ragnhildstveit E., Tveten Y., Berstad A. Laboratory handling of *Helicobacter pylori* critically influences the results of in-vitro metronidazole resistance determination. *Clinical Microbiology and Infection.* 2004; Volume 10, Issue 4, Version of Record online: 10.1111/j.1198-743X.2004.00779.x
 51. O'connor A., Taneike I., Nami A., Fitzgerald N., Murphy P., Ryan B., O'connor H., Qasim A., Breslin N., O'moráin C. *Helicobacter pylori* resistance to metronidazole and clarithromycin in Ireland. *Eur J Gastroenterol Hepatol.* 2010; 22(9):1123-1127.
 52. Shaman R., Niranga MD., Hithanadura J. de Silva. *Helicobacter pylori* Infection in Children. *Saudi J Gastroenterol.* 2009; 15(2): 86-94.
 53. Muhammad M., Ari F S., Iswan AN., Dadang M., Langgeng AW., Lukman HZ., Fardah A., Willy BU., David S., I Dewa NW., Jimmy BW., Alexander MJS., Fauzi Y., Syifa M., Pangestu A., Umami M., Hasan M., Yudith AAR., Phawinee S., Nasronudin DR., Rumiko S., Junko A., Yoshio Y. Surveillance of *Helicobacter pylori* Antibiotic Susceptibility in Indonesia: Different Resistance Types among Regions and with Novel Genetic Mutations. *PLoS ONE.*2016;11(12)
 54. Kim MM., Kim N., Lee SH. The effects of probiotics on PPI triple therapy for *Helicobacter pylori* eradication. *Helicobacter.* 2008;13:261-268.
 55. Rocco MZ., Gabriele B-P., Roberto F., Giovanni G., Enrico R., Franco B. Comparison of 1 and 2 weeks of omeprazole, amoxicillin and clarithromycin treatment for *Helicobacter pylori* eradication: the HYPER Study. *Gut.* 2007; 56(4): 475-479.
 56. Khurana R., Fischbach L., Chiba N . Meta-analysis: *Helicobacter pylori* eradication treatment efficacy in children. *Aliment Pharmacol Ther.* 2007;25:523-536.
 57. Malfertheiner P., Megraud F., O'Morain C . Current concepts in the management of *Helicobacter pylori* infection: The Maastricht III Consensus Report. *Gut.* 2007; 56(6):772-781.
 58. Miftahussurur M., Yamaoka Y. Diagnostic Methods of *Helicobacter pylori* Infection for Epidemiological Studies: Critical Importance of Indirect Test Validation. *Biomed Res Int.* 2016; Volume 2016, Article ID 4819423, 14 pages <http://dx.doi.org/10.1155/2016/4819423>
 59. Mohammed MK., Radwa RS., Ramy KA. *Helicobacter pylori*: A poor man's gut pathogen? *Gut Pathogens.* 2010; 2:2.
 60. Ramy KA., Mohammed MK., Radwa RS. Contaminated water as a source of *Helicobacter pylori* infection: A review. *Journal of Advance Research.* Volume 6, Issue 4.2015; Pages 539 - 547.