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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

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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-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

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RÉSUMÉ
Contexte : Helicobacter pylori a été associée à la diarrhée chronique, l'anémie, un retard de croissance, de cancers gastriques, ulcère gastro-duodénal, 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

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, dl = 2, P-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 premasticate 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 feaces 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 9years 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 diarrhoaea 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 diarrhoaea [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 require 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 endoscope 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 diarrhoiec 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 diarrhoiec 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^{BIOLINE} *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 POSITVE 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% O_2, 10%CO_2 and 85% N_2) using the anaerogen gas pak (Oxoid-England) at 37^0C 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 12^{th} 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 Testing:
The antibiotic susceptibility testing was done using the modified Kirby Bauer [44] technique using the disk diffusion method. Two to three colonies of the

H. pylori isolates were emulsified with physiological saline dispensed into sterile universal container. This suspension was standardised to 0.5MacFarland 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 37^0C [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 (m^2). 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).

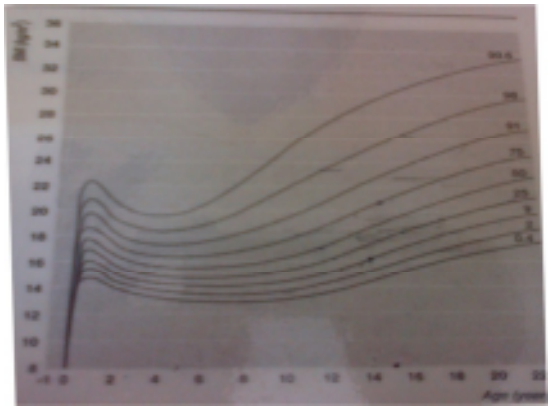

FIGURE1*:* BMI PERCENTILE GRAPH FOR CHILDRENBMI PERCENTILE GUIDE (Percentile) Underweight: Below 5; **Normal weight:** 5 – 85; **Overweight:** 86 – 96; **Obese:** 97- 99

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 (χ^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	3-5 persons	216	83.1
together at home	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 N=260	Attributes	Participants Number	Percentage
H. pylori stool antigen (HpSA) test	Positive	91	35.0
	Negative	169	65.0
Stool culture status	Positive (*H. pylori*-isolated)	26	10.0
	Negative (*H. pylori* not isolated)	234	90.0

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

N = 260	Culture of *H.pylori*		Total
H.pylori Ag. detection using HpSA	Number Positive (%) *(H.pylori isolated)*	Number Negative (%) *(H.pylori not isolated)*	
Number Positive (%) *(H. pylori Ag Present)*	3 (3.3)	88 (96.7)	91(100)
Number Negative (%) *(H. pylori 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

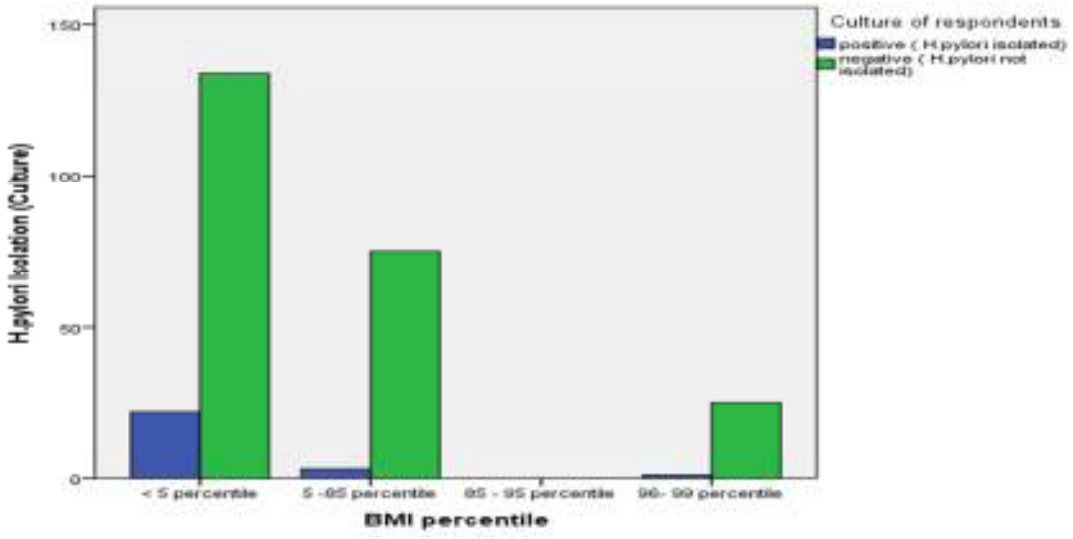

FIGURE 2: SHOWING CORRELATION OF *H. PYLORI* ISOLATION OF DIARRHOIEC CHILDREN AGAINST BMI PERCENTILE

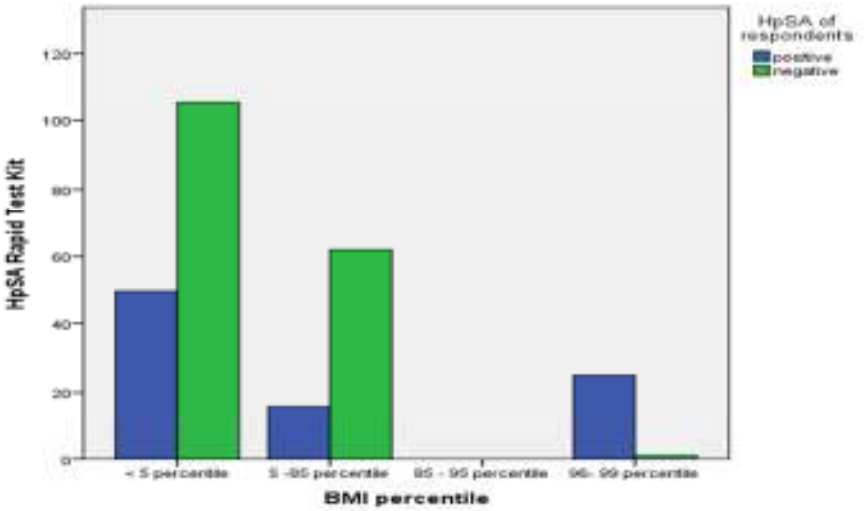

FIGURE 3: SHOWING HPSA RAPID ANTIGEN DETECTION TEST STATUS OF DIARRHOIEC CHILDREN AGAINST BMI PERCENTILE

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 Oyedeji *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.

Expatiating 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.

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