Prevalence of *Helicobacter pylori* in children by noninvasive stool Antigen Enzyme Immunoassay

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Abstract

Background: *Helicobacter pylori* (*H. pylori*) infection is contracted in childhood, and it is considered as an important risk factor for acid-pectic disorders and neoplasms later in life. A noninvasive *H. pylori* stool antigen test was used to determine the prevalence of *H. pylori* infection in children and the socio-demographic and clinical characteristic of children with *H. pylori* infection described.

Methods: A total of 102, symptomatic and asymptomatic, children aged 1-15 years, were consecutively recruited at the outpatient department of Plateau State Specialist Hospital in Jos, Nigeria. Eighty-seven (87/102, 85.3%) stool samples were analyzed using *H. pylori* stool antigen kit (HpSA™ GeneFronts elisaVUE TM, 2950 Scott Blvd, Santa Clara, CA 95054, USA) to detect *H. pylori* antigen. Prevalence of *H. pylori* infection, socio-demographic and clinical characteristics of children with *H. pylori* infection, and the association of these factors with *H. pylori* infection were determined.

Results: Of 87 stool samples tested, 32 were positive for *H. pylori* giving a *H. pylori* prevalence of 36.8%. Majority of the children were males (51.2%) and their median age (IQR) 10 (6-12) years. Majority of those with *H. pylori* infection resided in urban areas compared to rural areas (15, 51.7% versus 14, 48.3%); p = 0.354), lived in room type accommodation compared to flat apartment accommodation (17, 53.1% versus 15, 46.9%; p = 0.235) and fewer were HIV-positive compared to those who were negative (5, 15.6% versus 27, 84.4; p = 0.330). No significant associations were observed between any of the socio-demographic or clinical variables and *H. pylori* infection.

Conclusion In this study, the prevalence of *H. pylori* infection among the children tested was low. Stool antigen testing has the potential advantage of being relatively simple to perform and is also a non-invasive technique, therefore it could be a useful tool for mass screening for *H. pylori* infection in children.

Key words: *Helicobacter pylori,* Stool antigen, Enzyme immuno-assay, Prevalence, Children, Nigeria.

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Introduction

Helicobacter pylori (*H. pylori*) is a spiral-shaped microaerophilic gram-negative bacterium that colonizes the gastric mucosa and induces inflammatory response due to release of some cytotoxic substances.[1] Helicobacter Pylori plays a major role as an etiologic agent of peptic ulcer disease and a factor in the development of mucosa-associated lymphoid tissue (MALT) lymphoma of the stomach.[2,3] *Helicobacter pylori* infection is common in the community, especially in children and is widely transmitted from person-to-person through the feaco-oral route, oral–oral and gastro–oral.[4,5] Several factors have been associated with *H. pylori* transmission and includes; household overcrowding, poor sanitation, contaminated water sources and domestic animals.[2,6,7] The prevalence of *H. pylori* infection is higher in developing countries, ranging from 15%-69.7%[8-10] compared to developed countries where it is 1.2%-12.2%[11,12]

The diagnosis of *H. pylori* infection could be by invasive and non-invasive techniques. An invasive technique entails histology and culture where gastric biopsy is performed at endoscopy while non-invasive methods include the Urea Breath test (UBT), Polymerase Chain Reaction (PCR), simple serology and stool antigen assay [13]. The use of endoscopic biopsies has potential for sampling error, and simple serology examination does not reflect active infection, while UBT and polymerase chain reaction (PCR) requires expensive equipment and is laborious. Urea Breath Test (UBT) has been shown to have good performance, but may require exotic skills to perform.[14] The development of enzyme immunoassay that uses polyclonal captured antibodies (*Helicobacter pylori* stool antigen-HpSA™ GeneFronts elisaVUE ™, 2950 Scott Blvd, Santa Clara, CA 95054, USA), to detect *H. pylori* antigen in stool samples has been successful, convenient, and require little technical expertise to perform even in less equipped laboratories. However, the accuracy of stool antigen tests in different clinical settings outside of controlled studies poses a

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challenge. However, HpSA GeneFront's ELISA is the most widely studied and has shown to have acceptable performance in the detection of *H. pylori* infection.[15] Also, stool polyclonal and monoclonal antigen tests have been shown to be highly sensitive and specific even in children and HIV-1 infected patients globally[16,17] However, there is paucity of data about the use of enzyme immunoassay (EIA) test (HpSA) in resource-limited settings. The aim of this study was to determine prevalence of *H. pylori* in stool samples by HpSA test and to describe the socio-demographic and clinical characteristic of children attending an urban tertiary hospital in Jos, Nigeria.

Materials and Methods

Study design

A total of 102 children aged 2-15 years were consecutively recruited at the outpatient department of Plateau State Specialist Hospital (PSSH), Jos, Nigeria from January to April 2016. Children with or without symptoms of dyspepsia were included in the study. Children who had diarrhea, had received antibiotics, proton pump inhibitors as well as antacids in the preceding 3 weeks were excluded from the study.

Laboratory procedures

Fecal samples, not contaminated by urine, were collected using a clean universal container and stored at –20°C until analyzed.

The stool samples were processed and tested for *H. pylori* antigen in stool using the enzyme immunoassay (EIA) test (HpSA). This test utilizes a plurality of polyclonal anti-*H. pylori* captured antibodies adsorbed to micro-plate wells (GeneFronts ELISAVUE, USA). The stored stool samples were retrieved from the freezer, allowed to stand at 25°C on the bench for 30-45 minutes to assume room temperature and small pieces of stool (150mg) sample were then transferred into 1ml of sample treatment solution in a test tube and mixed thoroughly. One hundred (100) µl of treated sample was dispensed into the appropriate anti-body coated micro-wells, and incubated appropriately. The *H. pylori*-specific polyclonal antibodies conjugated, horseradish peroxidase were added, incubated, and washed before enzyme conjugate was added. Then 100 µl of Tetramethylbenzidine (TMB) chromogenic substrate was dispensed into each well, incubated for 15 minutes at room temperature, and stop solution added to stop reaction. A visible blue reaction indicated the presence of *H. pylori.* Absorbance at 450nm was measured with a spectrophotometer and the cut-off values of >20ng/ml was considered positive.

Statistical Analyses

Standard descriptive statistics were used to examine the prevalence, while association between each independent variable and the outcome (*H. pylori* infection) was examined using the Chi squared or Fisher's exact test for categorical variables. Variables that were associated with *H. pylori* infection with a P value of < 0.05 were considered statistically significant. All analyses were performed using Stata software version 10.0 (Stata Corporation, College Station, Texas, USA)

Ethical Clearance

An ethical clearance was granted for this study by the Health Research Ethics Committee of Bingham University Teaching Hospital and the children's caregivers gave their consent.

Results

Of the 102 subjects who were recruited, 87 (85.3%) had their stool samples analyzed. The overall prevalence of *H. pylori* infection among children whose stool samples were analaysed was 36.8% (32/87). Majority of the study subjects were males (51.2%) and their median (IQR) age was 10 (6-12) years (Table 1)

The prevalence of *H. pylori* infection was lower in children who were HIV positive (5; 15.6%) compared to those who were negative (27; 84.4%), though this difference was not statistically significant ($p = 0.333$). The prevalence of the infection was higher among children who were urban dwellers compared to rural dweller (51.7% versus 48.3%) and among children living in room type accommodation compared to those in flat apartment accommodation (53.1%, versus 46.9%), though this differences were not statistically significant, $p = 0.354$ and $p = 0.235$, respectively (Table 1).

There was no any significant associations between the socio-demographic or clinical variables and *H. pylori* infection (Table 1).

Table 1. Distribution and Socio-demographic Characteristics of Patients' associated with Helicobacter Pylori Infection

Characteristics	Total No Samples N (%)	H. Pylori Positive N (%)	H. Pylori Negative N (%)	P value
Age ≤5 (years)	18 (20.9)	7 (22.6)	11 (20.0)	0.788
Males	44 (51.2)	15 (48.4)	29 (52.7)	0.699
Caregivers' educational status				0.100
Illiterate	3 (3.9)	0 (0.0)	3 (6.5)	
Primary	22 (28.5)	13 (41.9)	9 (19.6)	
Secondary	35 (45.5)	11 (35.5)	24 (52.2)	
Tertiary	17 (22.1)	7 (22.6)	10 (21.7)	
Caregivers' Occupation				0.270
Unemployed	18 (20.7)	6 (18.7)	12 (21.8)	
Trader	37 (42.5)	15 (46.9)	22 (40.0)	
Civil servant	24 (27.6)	6 (18.8)	18 (32.7)	
Military	8 (9.2)	5 (15.6)	3 (5.5)	
Religion				0.715
Christian	54 (65.8)	19 (63.3)	35 (67.3)	
Muslim	28 (34.2)	11 (36.7)	17 (32.7)	
Residence				0.354
Urban	48 (58.5)	15 (51.7)	33 (62.3)	
Rural	34 (41.5)	14 (48.3)	20 (37.7)	
Accommodation type				0.235
Room	39 (44.8)	17 (53.1)	22 (40.0)	
Flat	48 (55.2)	15 (46.9)	33 (60.0)	
Water source				1.000
Well	8 (9.2)	3 (9.4)	5 (9.1)	
Borehole	44 (50.6)	16 (50.0)	28 (50.9)	
Tap	35 (40.2)	13 (40.6)	22 (40.0)	
Sanitary practice				0.990
Pit latrine	22 (25.9)	8 (25.8)	14 (25.9)	
Water closet toilet	63 (74.1)	23 (74.2)	40 (74.1)	
Keeping of animals/ pets at home				
Yes	35 (40.2)	11 (34.4)	24 (43.6)	0.396
Number of siblings				
≤4	58 (69.1)	22 (71.0)	36 (67.9)	0.771
Symptoms of dyspepsia	32 (37.6)	12 (38.7)	20 (30.0)	0.878
Positive HIV Status	18 (21.2)	5 (15.6)	13 (24.5)	0.330

Discussion

The results of this study showed the prevalence of *H. pylori* antigen in stool to be 36.8%. This prevalence was lower than the prevalence rate of 45% and 69.7% previously reported in some developing countries.[9,10] Our study's prevalence was similar to the 36.7% reported in one study from Lagos.[18] This study found a higher prevalence of *H. pylori* infection in females (51.6%) compared to males (48.4%), this was in contrast to a South African study where the prevalence was higher in boys (68%) than girls (64%).[19] In both of these studies, the Male to Female ratios were about the same 0.94 and 1.1 and there was no statistically significant associations between sex and *H. pylori* infection.

We observed that prevalence of *H. pylori* in HIV-positive children was 15.6%, which was lower than the 22.5% reported in a hospital-based survey among treatment-naïve HIV-infected Ugandan children.[20]

This study showed that *H. pylori* infection was higher among urban (51.7%) compared to rural (48.3 %) dwellers. Also, infection among those with room type accommodation, which is usually overcrowded, was higher (53.1%) compared to those with flat apartment accommodation (46.9%). Some urban residences and room type accommodations could be associated with overcrowding which is a proxy indicator of low socio-economic status. Low socio-economic status has been found to be associated with *H. pylori* infection.[12] The prevalence of *H. pylori* in children with symptoms of dyspepsia was 38.7% which was lower than the prevalence of 88% and 71% reported among Nigerian[21] and Kenyan adults[22] with dyspepsia, respectively.

We did not find any significant associations between any of the socio-demographic or clinical variables and *H. pylori* infection which might be attributed to our small sample size.

A limitation of this study was the small sample size, which may explain, the lack of statistical significance in the associations between some variables and *H. pylori* infection.

Conclusion

The prevalence of *H. pylori* infection was high among the children we studied. Though the prevalence of *H. pylori* infection was higher: in urban compared to rural children and room type accommodation compared to flat accommodation, these observed differences were not statistically significant, likely because of the small sample size of our study. We therefore recommend that as a line of future research in our environment, a larger study be conducted to determine the risk factors for *H. pylori* infection in children.

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