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Risk Factors Associated with *Helicobacter Pylori* Infections in Makurdi Northcentral Nigeria

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Abstract

Background: *Helicobacter pylori (H. pylori)* is a microaerophilic bacterium that inhabits the gastric mucosa of the human stomach. Infection with this bacterium leads to chronic gastritis, peptic ulceration, gastric cancers and gastric malt lymphoma. Studies have documented a higher prevalence in Africa and the transmission pathways are still vaque.

Methods: Eighty gastric biopsies were collected from the antrum of patients referred for endoscopy. Informed consent was obtained and questionnaires on risk factors such as type of water used, foods and habitswere administered to them. Genomic DNA was extracted from the tissue samples using ReliaPrep genomic DNA miniprep kit (Promega, Southampton UK), and *H. pylori* DNA was detected using a Singleplex PCR of the 16S rRNA gene.

Results: Of all the parameters analyzed, only involvement in milking of cows (OR=3.545, 95% CI: 2.488-5.052; p=0.029) and sharing of spoons and cups (OR=45.00, 95% CI: 8.769-230.936; p <0.001) had significant association with transmission, and patients were at increased risk of infection.

Conclusion: Transmission of *H. pylori* may be associated with the risk factors related to hygiene. Adequate hygiene is advocated to reduce the scourge of the infection.

Keywords: Risk factors, *Helicobacter pylori* infection, Transmission

Introduction

Helicobacter pylori are a Gram negative miroaerophic spiral bacterium which was discovered in 1983 [1]. It infects more than half of the world's population with prevalence ranging from 25% in developed countries to more than 90% in developing countries [2]. The increased risk of infection is especially high among those living in the developing world [3]. Infection with the bacterium causes chronic gastritis, peptic ulceration, gastric cancers and gastric Mucosa Associated Lymphoid Tissue (MALT) Lymphoma [3]. *Helicobacter pylori* have been rated as a "class one" carcinogen to the gastrointestinal tract by the World Health Organization [4]. It is in the same category as cigarette smoke is to lung cancer.

H. pylori prevalence and the rate of infection are inversely related to the standardof living and sanitary practice as revealed by a very high prevalence, especially in developing countries and lower socio-economic groups in the developed world [2,3,5]. The transmission pathways of *H. pylori* are still not clear. However, risk factors of transmission include precarious hygiene standards, over-crowding, contaminated environments and water sources amongst others [6]. Oral-oral, Feacal-oral, and direct contact modes have been proposed as possible modes of transmission, either with or without transitional transmission steps during episodes of diarrhea or gastro-oral contact in the event of vomiting [6]. It has been found that *H. pylori* can live in milk and water in its infectious bacillary form and inriver water for several months in a non-culturable but viable form [7]. Previous serological studies have related a high prevalence of antibodies against *H. pylori* among someprofessions (Abattoir workers, shepherds and veterinary workers) who are in direct contact with *H. pylori* infected animals [8]. *H. pylori* have also been isolated from the intestinal tract of dogs, cats and sheep [9].

H. pylori are believed to be transmitted primarily by faecal- oral and oral-oral routes, with water and food as possible vehicles of infection. However, exact modes of transmission are not easily determined because *H. pylori* are difficult to culture from environmental samples. There is some evidence for iatrogenic transmission through inadequately sterilizedendoscopes. *H. pylori* have been detected in vomitus, indicating the potential for gastro-oral transmission [10].

The burden of *H. pylori* infection is so much that the infected individuals live the rest of their lives taking drugs, avoiding certain foods and drinks because they believe it has no cure [11]. Although extensive research has been carried out on *H. pylori*, the data from Nigeria has tended to focus on its prevalence and very little on risk factors associated with *H. pylori* infection.

Our study was aimed at determining the association of risk factors with *H. pylori* infection in the study area. The specific objectives were to detect *H. pylori* from biopsies by PCR and to determine the relationship between *H. pylori* prevalence and risk factors in the study area.

Materials and Methods

Ethical approval

Ethical approval was obtained from the Health Research Ethics Committee of the Benue State University Teaching Hospital, Makurdi. All participants had medical referrals for gastric biopsy at the Department of Gastroenterology of the Benue State University Teaching Hospital, Makurdi. Volunteered participants were informed-consented with written consent. Subjects were patients who had various *H. pylori* associated dyspeptic symptoms including epigastric pain, fullness, vomiting, nausea and flatulence.

Sample size determination

Sample size was calculated using Raosoft (2014) Sample Size Calculator. At 0.05 alpha level of significance, 95% confidence level and a patient population size of 99 and previous prevalence 50%, a sample size of 80 was obtained.

Questionnaires

Validated Questionnaire containing questions on the risk factors associated with *H. pylori* infection was administered to volunteered participants.

Sample collection

A Consultant Gastroenterologist performed the endoscopy on informed- consented participants. Gastric biopsy samples were taken from the antrum of the patients into sterile McCartney bottles containing Brain Heart infusion broth and stored in the freezer at -200 C within 2 h of collection until transported to the Laboratory for analysis.

Extraction of genomic DNA

Genomic DNA was extracted from the tissue samples using ReliaPrep genomic DNA miniprep kit (Promega, Southampton UK). The Reliaprep uses spin columns that contain silica membrane for DNA purification. Briefly, about 200 µl of the macerated tissue materials in broth were dispensed into 2 ml Eppendorf tube containing 25 µl of proteinase K. The sample was mixed by gentle vortex and incubated for 5 min at room temperature. Then 200 µl of Cell Lysis Buffer was added and the sample vortexed for 10 seconds before incubation in a water bath set at 560°C for 10 min. Thereafter, 250 µl of Binding Buffer was added to the sample and mixed by repeated pipetting. The mixture was transferred to the Spin Column and centrifuged at 14000 rpm for one minute. The flow through in the collection tube was discarded. The column was washed by addition of 500 µl of Column Wash Buffer and centrifuged for 3 min at 14000 rpm. The washing was repeated twice. Columns were then placed into new Collection tubes and centrifuged at 14000 rpm for 1 min to remove residual Wash Buffer. Then 100 µl of Nuclease-free water was added into the columns, which were placed into 1.5 ml tubes, incubated for one minute at room temperature and centrifuged at 13000 rpm for one minute. DNA quality was checked by reading at 260/280 nm using Eppendorf Biophotometer Plus (Eppendorf, Germany). The DNA elute was labeled and stored in the fridge until required for testing.

Specific primers for detection of *H. pylori*

The primer sequences used were: 16S rRNA - F=GGAGGATGAAGGTTTTAGGATTG (23), 16S rRNA - R=TCGTTTAGGGCGTGGACT (18) (synthesized by Eurofins, Germany).

Detection of *H. pylori* 16S rRNA gene

H. pylori DNA was detected by using a Singleplex PCR that amplifies 294 bp fragment using a final primer concentration of 0.5 µM in a 25 µl reaction volume. The thermal profile comprised initial denaturation at 95°C for 3 min, followed by 35 cycles of 94°C for 30s, 70°C for 60s and 72°C for 60s and a final extension of 72°C for 5 min. All amplifications were carried out in Eppendorf Nexus Gradient Master Cycler (Eppendorf, Germany) using 2x PCR Master Mix from Promega (Southampton, UK). PCR products were electrophoresed at 100 V for 30 min using 1.5% agarose gel stained with Ethidium bromide.

Statistical Analysis

Data were analysed using Statistical Package for Social Sciences (SPSS) version 20, IBM Inc. Chi square was used to measure association; Odds Ratio OR was used to assess risk factors. Alpha level of significance was set at 0.05.

Case Group	Positive (%)	Negative (%)	Total (%)	Odds Ratio	95% Confidence Interval	X^2 value	p-value
Abnormal Mucosa (gastritis)	22 (73)	8 (27)	30 (100)	66.00	12.937-336.717	42.92	<0.001
Normal Mucosa	2 (4)	48 (96)	50 (100)				

Table 1: *Helicobacter pylori* Detected in Biopsies of Patients by PCR (n=80).

Results

Of the 80 biopsies collected, 30 cases with gastritis, 22 (73%) had *H. pylori* while 2 (4%) out of the 50 cases with normal mucosa had *H. pylori*. There was significantly increased risk of *H. pylori* infection in gastritis patients (Table 1). There was no significant association between the sources of water used and *H. pylori* infection in patients (Table 2). In Table 3, drinking of unpasteurized milk was associated with transmission of infection, which means those drinking unpasteurized milks are at increased risk of infection; drinking of alcohol had no significant association with transmission, eating of raw vegetables also had no significant association with transmission of the infection.

Of all the habits analysed, only involvement in milking of cows and sharing of spoons and cups had significant association with transmission, and patients were at increased risk of infection, the rest had no association (Table 4).

Sources of water	Number (%)	Number Positive (%)	Number Negative (%)
Well	24 (30)	8 (33.3)	16 (66.7)
Stream	9 (11.25)	4 (44.4)	5 (55.6)
Pipe borne	24 (30)	7 (29.2)	17 (70.8)
Bore hole	22 (27.5)	4 (18.2)	18 (81.8)
Rain water	1 (1.25)	1 (100)	0 (0)
Total	80 (100)	24	56
$\chi 2$=1.50, df=4, p=0.68			

Table 2: Distribution of *Helicobacter pylori* as Influenced by Sources of Water used by Patients in the Study.

Variables	Number (%)	Positive (%)	Negative (%)	Odds Ratio	95% Confidence Interval	X^2 value	p-value
Drinking of unpasteurized milk							
Yes	76 (95)	21 (27.6)	55 (72.4)				
No	4 (5)	3 (75)	1 (25)	0.127	0.013-1.293	4.06	0.044
Total	80 (100)	24	56				
Drinking of alcohol							
Yes	50 (62.5)	15 (30)	35 (70)				
No	30 (37.5)	9 (30)	21 (70)	1.076	0.254-2.176	0	0.1
Total	80 (100)	24	56				
Eating of raw vegetables							
Yes	59 (73.75)	18 (30.5)	41 (69.5)				
No	21 (26.25)	6 (28.6)	15 (71.4)	1.098	0.366-3.287	0.028	0.868
Total	80 (100)	24	56				

Table 3: Distribution of *Helicobacter pylori* as influenced by foods that are risk factors.

Discussion

The distribution of *H. pylori* as influenced by sources of water used in our study did not show any significant association between the sources of water used and *H. pylori* infection in patients (Table 2). This was not the case in Kazakhstan, where drinking river water had the highest risk of *H. pylori* infection (OR: 13.6, 95% CI=1.8–102.4; p<0.01), compared with tap water [12]. In South India, Almed et al. reported that *H. pylori* was higher in people with low clean water index (CWI) (88,2%) than those with higher CWI (33.3) [3]. Also, Ndip et al. reported that the very high prevalence of *H. pylori* in Africa might be linked to water sources [13]. Several other studies had highlighted the presence of the microorganism or their DNA in water [7,14-16]. The findings of this study did not also agree with the study by Papiez et al. that water-borne transmission of *H. pylori* could be an important source of infection in developing countries [8]. A study in Guatemala reported that *H. pylori* was unlikely to be transmitted by water [17] but epidemiological studies in many countries such as China [18], Lima Peru [19], and Colombia [20] had shown that infection was related to *H. pylori* contaminated water sources. Dube et al. also reported that *H. pylori* could live in tap water in its infectious bacillary form, and in river water for several months in the non-culturable but viable cocoid form. According to Sorbey et al., faecally contaminated water had the potential for transmission *via* the faecal-oral route [21]. Faults in pipelines near or across a drainage system were found to be strongly correlated with high level of contamination of pipe-borne water supplies in Lagos, Nigeria. Goodman et al. and Steinberg et al. reported in the US that the presence of *H. pylori* in untreated well correlated with infection in consumers [22,23]. In Japan, *H. pylori* DNA was detected in well water used by persons who had acquired *H. pylori* infection in the past [24]. In continuation, Eguari and Aboaba detected *H. pylori* DNA in well water obtained from all five wells from which five seropositive members had drunk [25]. Ahmed et al. in South India also reported that the prevalence of *H. pylori* infection among people who drank well water was 92% compared with 74.8% of those who drank tap water (p<0.001) [3]. Several Epidemiological studies in many countries such as rural China, Lima Peru and Colombia had also shown that infection was related to *H. pylori* contaminated water sources [18-20]. However, in agreement with our study, Klein et al. reported in a study in Guatemala that *H. pylori* were unlikely to be transmitted by water [17].

Habit	Response number (%)	Positive (%)	Negative (%)	Odds Ratio	Confidence Interval 95%	X^2 value	P-value
Keeping of domestic animals							
Yes	70 (87.75)	21 (30)	49 (70)				
No	10 (12.25)	3 (30)	7 (70)	1	0.236-4.246	0	0.1
Total	80 (100)	24	56				

Washing of hands after defecation							
Yes	79 (98.75)	24 (30.4)	55 (69.6)				
No	1 (1.25)	0 (0)	1 (100)	0.696	0.602-0.805	0.43	0.51
Total	80 (100)	24	56				
Keeping of long nails							
Yes	19 (23.75)	5 (26.3)	14 (73.7)				
No	61 (76.25)	19 (31.1)	42 (68.9)	0.789	0.248-2.508	0.16	0.69
Total	80 (100)	24	56				
Involvement in milking of cow							
Yes	2 (2.5)	2 (100)	0 (0)				
No	78 (97.5)	22 (28.2)	56 (71.8)	3.545	2.488-5.052	4.79	0.029
Total	80 (100)	24	56				
Sharing of spoons and cups							
Yes	17 (21.25)	15 (88.2)	2 (11.8)				
No	63 (78.75)	9 (14.3)	54 (85.7)	45	8.769-230.936	34.86	<0.001
Total	80 (100)	24	56				
Sharing of bedroom							
Yes	72 (90)	22 (30.6)	50 (69.4)				
No	8 (10)	2 (25)	6 (75)	1.32	0.247-7.061	0.1	0.75
Total	80 (100)	24	56				
Sucking of thumb in childhood							
Yes	20 (25)	6 (30)	14 (70)				
No	60 (75)	18 (30)	42 (70)	1	0.331-3.017	0	0.1
Total	80 (100)	24	56				
Sharing of tooth brush							
Yes	2 (2.5)	1 (50)	1 (50)				
No	78 (97.5)	23 (29.5)	55 (70.5)	2.391	0.143-39.887	0.39	0.53
Total	80 (100)	24	56				
Washing of fruits before eating							
Yes	78 (97.5)	23 (29.5)	55 (70.5)				
No	2 (2.5)	1 (50)	1 (50)	0.418	0.025-6.975	0.39	0.53
Total	80 (100)	24	56				

Table 4: Distribution of *Helicobacter pylori* as Influenced by Habits that are Risk Factors.

Involvement in milking of cows had significant association with transmission of the infection (Table 4). This agreed with the report of Papiez et al. who showed a higher prevalence of antibodies against *H. pylori* among some professions (Abattoir workers, Shepherds and Veterinarians) probably due to direct contact with *H. pylori* infected animals [8]. Papiez reported that milking appears to be a possible

means of transmission. Bragonca et al. found that *H. pylori* could live in milk in its infectious bacillary for several months in a non-culturable but viable form [7]. It has also been reported that *H. pylori* could live for several days in milk in its infectious bacillary form [5].

Sharing of spoons and cups had a significant association with transmission of *H. pylori* infection in our study as indicated in Table 4. Sharing of spoons, cups, premastication for young children, sharing water for bathing and washing hands and limited sanitary facilities have also been shown to be associated with increased prevalence of the organism [13,3].

In this study, there was no association between *H. pylori* and sharing of bedrooms (Table 4). This did not agree with the findings of Farrel et al. who reported that sharing a bed or bedroom with an infected sibling at the age of 3 significantly increased the risk of childhood *H. pylori* infection [26].

In Table 3, drinking of alcohol was not associated with *H. pylori* infection in our study. This agreed with the findings of Mckeown et al. who reported that antibody status did not differ with respect to alcohol [18]. This did not however agree with the findings of Zhang et al. who reported an association between the two in Australia. They found that alcohol consumption and pathology active gastritis were associated with *H. pylori* infection. Active gastritis was associated with alcohol consumption. They therefore concluded that alcohol consumption was associated with *H. pylori* infection [27]. Ogihara et al. also reported that drinkers had a 0.88 (0.74-0.91) fold greater risk of *H. pylori* seropositivity than those who had never taken alcohol [28]. However, Brenner et al. reported that alcohol had strong antimicrobial activity and stimulates gastric acid secretion and might therefore compromise living conditions of *H. pylori* in the stomach. They assessed the relationship of alcohol consumption with *H. pylori* infection among 1,785 participants aged 18-88 years in the German National Health and Nutrition Survey. There was a clear, intense dose-response relation between reported alcohol consumption and *H. pylori* infection. The adjusted prevalence ratios (95% confidence intervals) for *H. pylori* infection among persons who consumed up to 10, 10-20, and more than 20 gm of alcohol per day compared with non-drinkers were 0.93 (0.77-1.13), 0.82 (0.65-1.04), and 0.71 (0.55-0.92) [29]. These findings supported the hypothesis that moderate alcohol consumption might facilitate spontaneous elimination of *H. pylori* infection among adults.

Several studies have shown a possibility of transmission pathway through domestic cats, dogs and sheep [8]. However, this study did not find any association between keeping of domestic animals and *H. pylori* infection (Table 4). The findings were in concordance with the findings of Webb, who reported in the United Kingdom that there was no association between *H. pylori* seropositivity and cat ownership during childhood [30]. In Southern Germany, in 1966-1997 among children in first grade, neither contact with specific kinds of animals was positively associated with *H. pylori* infection [31].

Washing of hands after defecation was not associated with *H. pylori* infection in the present study as seen in Table 4, but in China, prevalence was elevated due to infrequent hand washing before meals. Washing less than half of the time (OR=1.6, 95% CI: 1.0-2.5; % of seropositivity=74.4%), and never washing (OR=3.8, 95% CI: 1.5-31.0; % seropositivity=87.5% [32].

Conclusion

H. pylori infection in the study was found not to be associated with sources of water used, drinking of alcohol, eating of raw vegetables, keeping of domestic animals, keeping of long nails, sharing of bedroom, sharing of tooth brush and sucking of thumb in childhood rather associated with sharing of cups and spoons and involvement in milking of cows.

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Conflicts of Interest

Authors declared no conflicts of interest.

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