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Prevalence of Virulence Genes and Associated Risk Factors of *Helicobacter pylori* Infection Among Adults in Gastric Cancer Risk Region of North Central, Nigeria

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Abstract: *Introduction*: *Helicobacter pylori (H. pylori*) is the causative agent of chronic gastritis and peptic ulcer diseases with associated risk factor of developing gastric cancer and mucosal-associated lymphoid tissue (MALT) lymphoma if left untreated. In this study, we evaluated the prevalence of virulence genes and associated risk factors of *H. pylori* among adults in a gastric cancer risk region of Nasarawa State, North central Nigeria. *Methods*: This was a descriptive cross-sectional study to determine *H. Pylori* status of 434 adults in gastric cancer high risk region of Nasarawa State from January to August, 2021. Associated risk factors and virulence genes (positive/β-(1, 3) galT of jhp0562, cagA, vacA, and hrgA) was evaluated from gastric biopsy specimens of dyspepsia patients. *Results*: The overall prevalence of *H. pylori* infection was 45.6% (198/434). The rate of infection was higher in male subjects (40.6%) than in female (23.8%); P<0.001) and higher significantly among adults with the following associated risk factors: unboil water, consumption of alcohol, low income, and Urban residency. Unboiled water consumption and alcohol consumption were independent risk factors for *H. pylori* infection (odds ratio=7.48 vs OR=9.78 respectively). Of the 198 strains cultured, 76.9% carried Western-type cagA, with a higher proportion in Male (86.4%) than in Female (50.0%), P=0.05). Patients infected with East Asian-type cagA strains (P=0.027) have lesser inflammation scores in the antrum than those infected with the Western-type cagA strains. *Conclusion*: Our study revealed a high prevalence of *H. pylori* infection in Nasarawa State, with unclean water source, and alcohol consumption as significant risk factors for *H. pylori* infection. The incidence of gastric cancer in Nasarawa State is associated with circulating virulence genes of cagA, vacA, hrgA and jhp0562-positive/β-(1, 3) galT.

Keywords: Virulence Genes, Prevalence, Risk Factors, *Helicobacter pylori*, Infection, Gastric Cancer, North Central, Nigeria

1. Introduction

Helicobacter pylori (*H. pylori*) infection is the commonest human infection worldwide accounting for about half (50%) of the world's populations being infected with the bacterium. *H. pylori* has been implicated as an etiological agent responsible for a spectrum of diseases in the gastrointestinal tract (GIT) notably, gastric adenocarcinoma, gastritis and peptic ulcer [1]. Robin and Barry Marshall identified and isolated *H. pylori* from the human stomach, also drew inference to the strong association between *H. pylori* implication in gastritis, peptic ulcers, and gastric mucosa-associated lymphoid tissue lymphoma. [1, 2]. In low income countries where poor hygiene, lack of portable water, overcrowding as a mainstay, 60%–80% of the population

harbor *H. pylori* during childhood age, conversely in developed countries, the prevalence rate is low ranging from 20% to 50% [3]. In Nigeria, the prevalence rate is high as 87% [4], while studies from neighboring African countries revealed similar prevalence rate of 91.7% in Egypt, [5] 97% in Gambia, [6] and 75.4% in Ghana. [7] Similarly, in Asia, prevalence rates of 92% have been reported in Bangladesh [8] and 62% prevalence was found in Chinese [9]. Increased risk of *H. pylori* is associated with several environmental factors, which include overcrowding, poor personal hygiene, and low socioeconomic factors among others [10]. With improved sanitary condition and standard of living, decrease in prevalence rate of *H. pylori* infection is observed [10]. Bacterial host factor plays a key role in gastric cancer development in addition to fermented food, salt, and alcohol consumption [11]. Precancerous processes that mimic chronic mucosal inflammation are believed to be associated with *H. pylori* virulence factors with prolonged disease outcome and severity in patients [12]. Increase risk to intestinal metaplasia, gastritis, peptic ulcer, and gastric cancer are associated with *H. pylori* strains with cagA- genes [13]. There is glaring difference between Western-type CagA and East Asian-type CagA in the CagA sequences of the second repeat of the C-terminal region. The binding affinity of the East Asian-type CagA to the Src homology-2 domain-containing phosphatase 2 (SHP2) region results in a greater risk of peptic ulcer and subsequent development of gastric cancer in comparison to Western-type CagA [14]. Furthermore, studies have revealed deletion in the 39-bp of strains isolated in East Asia CagA [15]. Predictor associated to disease severity has been linked to either s or m region of the VacA c region 30 end of the polymorphic site [16]. The presence of jhp0562 and β-(1,3) galT is link with peptic ulcers development [17, 18]. There's need to generate evidence based data to inform policy makers and to serve as a guide for improved treatment [19].

In Nigeria, CagA and VacA virulence genes producing strains of *H. pylori* has been showed to be in circulation and the proteins expressed in Gastric cancer disease outcomes. Resistance to antibiotics can reach as high as 100% due to treatment failure; this is because there is no standard treatment regimen for *H. pylori* in Nigeria which increase burden of disease. To have a clear understanding of *H. pylori* burden in Nasarawa state, we aimed to determine the associated risk factors, virulence genes, prevalence of *Helicobacter pylori* infection among Adults in gastric cancer risk region of Nasarawa State.

2. Methods

Study design: A total of 434 participants across the 13 local government area of Nasarawa State were enrolled for this study. A well-structured questionnaire was administered to participants following informed consent. Tissue biopsy was collected by an experienced endoscoptist around the pylori ring approximately 2–3 cm at the antrum and second samples around the corpus curvature. Biopsy samples for bacterial culture were placed on a transport medium and then placed on a mobile freezer at -20˚C and transported to Federal Medical Center Keffi Histopathology Department for analysis.

Determination of *H. pylori* infection status: To determine the *H. pylori* infection status, an immunohistochemistry (IHC) was conducted to confirm *H. pylori* diagnosis by histology and culture. In undertaking culture, we checked for the presence of *H pylori* from one antral biopsy specimen which was homogenized and inoculated into a *H. pylori* selective media (Nissui Pharmaceutical co., LTD, Tokyo, Japan). Each of the antral and corporal specimen from each patient was used for histological examination. According to updated Sydney classification system we classified bacteria density, atrophy, intestinal metaplasia and degree of inflammation into four grades namely; normal-0, mild-1, moderate-3 [20]. A positive was considered grade 1. Operative Link on Gastritis Assessment (OLGA) system was used to grade gastritis stage and to determine and assessed based on their antrum or corpus location of atrophy [21].

H. pylori Culture and Isolation: Columbia blood agar base plates were used aseptically under Biosafety Cabinet (BSC II) (Thermo Scientific) where Biopsy specimens were rolled over the surface of the plate. The medium (Oxoid CM0331 agar) was supplemented with 1% vitamin mix (Isovitalex), 7% horse serum (Oxoid SR0048) and an *H. pylori* selective supplement (Dent, SR0147E Oxoid) comprising of trimethoprim (2.5mg), amphotericin B (2.5mg), vancomycin (5.0mg), and cefsulodin (2.5mg). At an atmosphere of 85% N2, 10% CO_2 and 5% O_2 for 4–10days the plates were incubated at 37°C.

Molecular analysis: Upon total genomic DNA extraction from biopsy samples using QIAamp DNA Mini Kit; Qiagen, Hilden, Germany, PCR was performed using 16S RNA specific primers for *H. pylori* under the following conditions; Initial denaturation at 95°C for 5 mins and 35cycles at 95°C for 30s, 54°C for 30s and 72°C for 30s and a final extension time of 72°C for 10min. Samples were considered positive when the visible band was the same size as that of the positive control DNA. The primer for 110bp product of the 16SrRNA sequence represented by the forward primer sequence: 5′-CTGGAGAGACTAAGCCCTCC-3′ and the reverse one: 5′-ATTACTGACGCTGATTGTGC-3′.

Virulence genes (cagA, vacA, jhp0562/β-(1,3)galT and hgrA) were detected using PCR specific primers (Table 1) with the same amplification conditions and assay protocol as earlier described.

Statistical Analysis: All data were cleaned and checked for completeness in excel version 2019 before they were exported to Epi-info version 7.1. Descriptive analysis were done and presented as tables. Discrete variables (frequency of occurrence of *H. pylori*, demographics, associated risk factors, and sanitation status) were tested using frequency and chi-square test. A multivariate logistic regression model was used to calculate the odds ratios (OR) of the clinical.

Table 1. *The primers used for detecting virulence factors of H. pylori infection.*

Target gene	Control strains	Primer pair (5′-3′)	Amplicon length (bp)	PCR conditions
cagA	J99	F: GATAACAGGCAAGCTTTTGA R: CTGCAAAAGATTGTTTGGCA	499	94°C, 1 min; 52°C, 1 min; 72°C, 1 min (35 cycles)
vacA	J99/Tx30a	F: ATGGAAATACAACAAACACAC R: CTGCTTGAATGCGCCAAAC	259 (s1)/286 (s2)	94°C, 1 min; 52°C, 1 min; 72°C, 1 min (35 cycles)
hrgA	J99/Tx30a	F: TCTCGTGAAAGAGAATTTCC R: TAAGTGTGGGTATATCAATC	594	94°C, 1 min; 56°C, 1 min; 72°C, 1 min (30 cycles)
jhp0562/β-(1,3)galT	P12/J99	F: TGA AAA GCC CTT TTG ATT TTG R: GCT GTA GTG GCC ACA TAC ACG	301/602	95°C, 30 s; 56°C, 30 s; 72°C, 30 s (35 cycles)

Table 2. *Socio-demographic characteristics, Frequency (%) and mean (SD) of characteristic variables by H. pylori seropositivity status of participants in Nasarawa State, North Central, Nigeria (n=434).*

Variable	Total number examined	*H pylori* seropositivity	
		Positive No (%)	Negative No (%)
Age (Yrs)			
<20	45	21 (46.7)	24 (53.3)
21-30	75	44 (58.7)	31 (41.3)
31-40	110	50 (45.5)	60 (54.5)
41-50	100	60 (60.0)	40 (40)
>50	104	75 (72.1)	29 (27.9)
Sex			
Male	234	155 (66.3)	79 (33.7)
Female	200	95 (47.5)	105 (52.5)
Residency			
Urban	194	100 (51.5)	94 (48.5)
Rural	240	150 (62.5)	90 (37.5)
Water Source			
Borehole	140	70 (50.0)	70 (50)
Well	84	60 (71.4)	24 (28.6)
Stream	210	120 (51.7)	90 (48.3)
Educational			
No	135	75 (55.6)	60 (44.4)
Primary	45	20 (44.4)	25 (53.6)
Secondary	120	85 (70.8)	35 (69.2)
Tertiary	134	70 (52.2)	64 (47.8)

3. Results

PCR and Culture analysis: Out of the 434 participants enrolled in the study, biopsies of either antrum or corpus or both biopsies origin of 215 were positive for *H. pylori* through culture analysis. One hundred and ninety-eight (198) were positive by PCR giving an overall prevalence of 45.6% (198/434). Of the 198 isolates, 30 (15.15%) were obtained from biopsies of antrum origin, while 168 (84.85%) were from corpus tissues. Antrum prevalence by PCR was 45.6% (198/434).

Sociodemographic characteristics and *H. pylori* positivity status: Out Of the 434, 155 (35.7%) females and 95 (21.9%) males were positive for *H. pylori*. The age range was between 20 and above 50years, with a mean age of 20.7±15.5years, and 25% (110/443) of subjects were between 21 to 50years. Residency plays a key role in determining *H. pylori* seropositivity with 62.5% (150/434) were positive to *H. pylori* are urban dwellers with 51.7% (120/434) had stream as their source of water with 55.6% (75/434) had no any form of formal education.

Endoscopic conditions and Virulence gene detection in *H. pylori* positive individuals: Endoscopy grading was performed in 434 cohorts that are enrolled in the study showing Vac A genes having the highest seropositivity to *H. pylori* along the endoscopic grading of 36.4% in normal mucosa, 30 (40,5%) in peptic ulcer and 55.5% is gastritis patients. The highest number of *H. pylori* positives [180 (76.9%)] was obtained in pan gastritis patients, followed by participants with peptic ulcer 31 (13.2%). No *H. pylori* isolate was obtained in participants with gastric cancer. Virulence genes were determined for all confirmed *H. pylori* positive patients obtained from culture isolates and biopsy DNA out of which the cagA gene was detected in 62% (145/234), dupA in 53.4% (125/234), vacAs1 in 66.2% (155/234), vacAs2 in 20.1% (47/234), vacAm1 in 69.2% (162/234) and vacAm2 in 21.4% (50/234) strains.

The cagA virulence gene was highest [111 (76.5%)] in pan gastritis patients followed by individuals with peptic ulcer [17 (11.7%)], while participants with hemorrhages and polyps had the lowest cagA detection [2 (1.4%)]. Virulence genes cagA, dupA, vacA s1/m1 were detected in all disease conditions except gastric cancer where *H. pylori* was not found. Detection of vacA s1 was significantly high 26 (16.8%) in patients with peptic ulcer disease (P=0.036). Similarly, vacA s1/m2 was considerably high in patients with gastric erosion (P=0.0029) (Table 3). Overall, 90.6% (212/234) of *H. pylori* strains were positive for vacA genotypes (data not exclusive but form unions and intersections).

Association between cagA, dupA and vacA genotypes in *H. pylori* strains: Table 4 shows the association between cagA, dupA and vacA genotypes in *H. pylori* strains. The vacA s1 was found to be significantly high (P=0.000) in the cagA and dupA positives patients. Similarly, vacA m1 positive individuals recorded a significantly high cagA 123 (75.9%) and dupA 100 (61.7%) genotypes. Furthermore, there was a high detection rate of cagA 82.6% (109/132) and dupA 63.6% (84/132) in the vacA positive patients. The rest of the vacA genotypes were not significantly high in cagA and dupA positive individuals.

Table 3. *Socioeconomic and epidemiological risk of H pylori.*

Characteristics	Helicobacter pylori seropositivity		
	OR	95% CI	p-value
Age (Yrs.)			
<20	1.34	(0.51–2.65)	>0.99
21-30	1,56	(1.45–1.65)	
31-40	2.45	(2.72–3.76)	
41-50	1.98	(1.77–2.11)	
>50	6.77	(5.44–9.66)	
Sex			
Male	1.54	(0.34–2.65)	0.35
Female	4.67	(3.61–5.90)	
Residency			
Urban	1.23	(1.33–1.76)	0.24
Rural	1.66	(0.44–3.65)	
Water Source			
Borehole	2.56	(2.33–4.55)	4.24
Well	2.45	(3.33–4.34)	
Stream	2.98	(2.34–4.56)	
No. of family members			
1	3.56	(3.33–4.87)	0.48
2 to 4	4.44	(4.56–5.67)	
>5	4.75	(0.45–8.75)	
Alcohol Consumption			
Yes	1.34	(0.78–1.90)	0.34
No	1.43	(0.61–1.65)	

Table 4. *Detection and Distribution of H pylori and its virulence genes in patients with various gastroduodenal disease outcome.*

	Normal mucosal	Peptic ulcer	Gastric adenocarcinoma	Gastritis	Total (%)
	n=223 (%)	n=125 (%)	n=50 (%)	n=36 (%)	
H. pylori positive	n=11	n=74	n=50	n=63	n=198
VacA	4 (36.4)	30 (40.5)	21 (42.0)	35 (55.6)	90 (45.5)
cagA	2 (18.20	25 (33.8)	20 (40.0)	20 (31.7)	67 (33.8)
hrgA	4 (36.4)	10 (13.5)	7 (14.0)	5 (7.9)	26 (13.1)
Pre-EPIYA	1 (9.1)	9 (12.2)	2 (4.0)	3 (4.7)	15 (7.6)

4. Discussion

An overall prevalence rate of 45.6% was reported in this study, which is relatively low compare to other parts on Nigeria and developing nations where higher prevalence rate of 80 to 90% has been recorded [2, 28]. However, South Africa records a lower prevalence rate of 50.6% [5]. Furthermore studies in the following countries Iran, Indonesia, Thailand reported lower prevalence rate [22-24]. Allelic diversity and some degree of genomic variations have been seen in *H. pylori*, this special features plays a pivotal role in gastric cancer development in patients globally and virulence factors are responsible for this [25]. Our study reveals the role of virulence genes in gastroduodenal disease development. The *vac*A and *cag*A are responsible for inducing mucosal associated lymph tissue (MALT)-lymphoma, gastric adenocarcinoma and peptic ulcer disease (PUD) in patients [26]. While dupA is associated with duodenal ulcer [27]. Our study reveals the degree of severity and frequency of gastroduodenal disease varies geographically as it relates to *H. pylori* [28, 29]. The distribution of this virulence marker pattern in circulating strains has been reported in strains harboring repeated EPIYA segment sequence A-B-D and cagA East-Asian-type resulting in higher risk of gastric cancer and peptic ulcer [30]. Studies have revealed low incidence of peptic ulcer and gastric cancer in population with high percentage of vacA m2, dupA negative and cagA negative *H. pylori* strains [31].

The most studied virulence genes of *H. pylori* are a toxic protein called CagA which is found on the cag-PAI. Strains with *cag*-PAI are more pathogenic than none-*cag*-PAI strains [38]. Our study presented a prevalence rate of 62% cagA in positive strains which concurred to similar studies reported in Morocco and Tunisia which are 62,7% and 63.1% respectively [32, 33]. Conversely, there was a relatively low prevalence rate reported in South Africa with 90% cagA positive strains were reported [34] while in some studies in Gauteng, 87% cases of cagA positive were found among asymptomatic children age between 6 and 15years. Globally, in Taiwan, 83% cagA positive strains were found in isolates from patients with chronic gastritis and peptic ulcer [35]. Also, 93% in Nigeria [36] and 96% in Indian [37] and 85% cagA was reported among Alaskans (USA) [38]. Among Turkish patients with dyspepsia, 74% cagA was detected [39]. Conversely, a lower prevalence of *cag*A has been documented in countries such as Cuba [40], Pakistan, Egypt, Israel and Jordan [41].

The Vacuolating cytotoxin (vacA) which is a pore-forming toxin secreted through an auto transporter has been pivotal in

gastroduodenal disease progression. The gene employs the toxigenicity mechanism of binding to the receptors of the eukaryotic sphingomyelin lipid cells. The gene induces apoptosis by targeting large intracellular vacuoles and the mitochondria. Studies have revealed polymorphisms in VacA with a divided signal of intermediate, middle and deletion regions. Of all the allelic combinations, the vacA s1/m1 alleles are the most virulent, while the s1/m2, s2/m1 and s2/m2 genotypes demonstrate little to no pathogenicity. Our study showed vacA s1 to be predominant, which is similar to findings reported elsewhere such as Eastern Cape South Africa [43], Thailand [44] and Indian [45]. In the same vein, Findings in Alaska (USA) [46], Cuba [47] and Morocco [48] showed the dominance of vacAs1 subtypes and its link with disease status. On the other hand, a lower prevalence of s1 type allele has been reported in Jordan [49] as well as Iran [50].

From our studies, our findings revealed a high prevalence of VacA (90.6%) and cagA (90.6%) which make patients positive to *H. pylori* at risk of duodenal disease progression. VacA genes is absence in 30 strains which could be as a result of adverse stomach acidic conditions [51]. Numerical data of vacA polymorphs differ from strains due to genetic composition and geographical location of the organism. For example, m1 genotype appears more than m2 in African population while the two subtypes are almost equally distributed within Europe and Latin America [52]. This result is comparable to a study conducted in Nigeria is lower in terms of the presence of cagA and vacA virulence genes among *H. pylori* strains [54]. However, no correlation with pathology could be observed in the Nigerian study contrary to our findings which showed a significant relationship between vacAs1 and peptic ulcer as well as vacAs1/m2 and gastric erosion. The reason probably could be the influence of host genetic make-up and environmental conditions [55]. In the same vein, our finding is parallel with a study conducted in Brazil which showed that vacA s1/m1 genotype may be considered an important virulence factor in the development of gastric diseases [56].

5. Conclusion

Our study revealed high prevalence rate of *H pylori* infection in Nasarawa State North central, Nigeria revealing its association with gastric cancer incidence. We revealed the importance of *H. pylori* infection and the role of effector protein CagA in gastric disease development. The strains with intact cagPAI mediating efficient CagA translocation are important for the commencement of pathogenicity. CagA, after its translocation and tyrosine phosphorylation, dysregulates the cellular signaling pathways, altering the function of several cellular proteins. Therefore, the CagA-mediated altered activity of several cellular proteins inhibits apoptosis and induces cellular proliferation.

Limitations

The Data analyzed in this study is for a sub-population, and may influence the generalizability of these results, compared in a larger population.

Authors Contribution

A. A and A. I. A designed the study; prepared the isolates; acquired, analyzed, and interpreted the data; and wrote the manuscript. R. G contributed to study design and acquired the data. A. A, A. C. O, R. P. G, A. I. A, acquired data. A. A and A. I. A acquired data and prepared the isolates. R. M contributed to data analysis and interpretation. A. I. A, A. C. D, R. P. G, A. C. O and A. A. A contributed to manuscript revision for scientific content. All authors read and approved the final manuscript.

Ethical Consideration

Ethical approval was obtained from the Ethical Review Board of Federal Medical Center, Keffi. Prior to enrollment in the study, all participants were informed as consent on the objectives and background of the study. Information was provided toward the risks and benefits of the current study. Similarly, a designated questionnaire and data were collected after obtaining returned informed consent. Anonymity and confidentiality of the participants were maintained.

What Is Already Known on This Topic

1) *Helicobacter pylori* has been implicated as an etiological agent responsible for a spectrum of diseases in the gastrointestinal (GI) tract.
2) Increased risk of *H. pylori* is associated with several environmental factors which include overcrowding, poor personal hygiene, and low socioeconomic factors among others.

What This Study Adds

1) Our findings revealed a high prevalence of VacA (90.6%) and cagA (90.6%) which make patients positive to *H. pylori* at risk of duodenal disease progression.
2) We revealed the importance of *H. pylori* infection and the role of effector protein CagA in gastric disease development.

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