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Distribution of *vacA* and *cagA* status of *Helicobacter pylori* from endoscopy referral patients of Benue State University Teaching Hospital Makurdi, Benue State Nigeria

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Abstract

Helicobacter pylori (*H. pylori*) infection is a global health issue, with different prevalence and virulence factors among diverse populations. There is not much information available regarding the prevalence of the genotypes of *H. pylori* isolates in Makurdi, Benue State. This study aimed to determine the distribution of *vacA* and *cagA* status of *H. pylori* among patients referred for endoscopy at Benue State University Teaching Hospital, Makurdi. A total of eighty (80) patients undergoing endoscopy were enrolled in the study. Gastric biopsy samples were collected and DNA was extracted for molecular analysis. *H. pylori* was detected using polymerase chain reaction (PCR) targeting the 16S rRNA gene. Subsequently, the *vacA* and *cagA* genotypes were identified through specific PCR assays. We found that *H. pylori* alleles of signal and mid regions of *vacA* detected in biopsies showed that s1 had the highest frequency of 24 (100%) followed by s1c 22 (92%) while the least were s2, s1+s2 and m1+m2 (frequency=1; 4%) each. s1a was not detected. The distribution of *H. pylori* genotypes detected in biopsies revealed that s1+m2 had the highest frequency of 22 (92%) followed by s1c+m2 19 (79%). The least were s2+m1, s1c+m1+m2 and s1c+s2+m2 1 (4%) each. Seven (29%) of the positive cases were *cagA* positive, 71% of the *cagA* positive were s1c+m2. The distribution of *vacA* s and m alleles and *cagA* status from *H. pylori* infected patients indicated that s2 and m1 all had the highest *cagA* positives (100%) each and m2 had the lowest *cagA* positives (22.7%). The distribution of *vacA* genotypes and *cagA* status from *H. pylori* infected patients showed that s1c+m1+m2 and s1+s2+m2 all had *cagA* (100%) each and s1c+m2 had the least (26.32%) frequency of *cagA*. This study reported for the first time the prevalence of *H. pylori* genotypes in patients with gastrointestinal complaints in Makurdi and contributes valuable insights into the molecular epidemiology of *H. pylori* in the Benue State, throwing more light on the prevalence and virulence factors that may influence disease. Further studies are needed to understand epidemiological importance of the genotypes of *H. pylori* isolates in this region and the association between the virulence genes and clinical outcomes.

Keywords: *Helicobacter pylori*; Prevalence; Patient; Genotypes; PCR; cagA

1. Introduction

Helicobacter pylori (*H. pylori*) is a Gram-negative bacterium that colonizes the human gastric mucosa, and it has been linked to various gastrointestinal ailments such as gastritis, peptic ulcers, and gastric malignancies [1,2]. The complexity of *H. pylori*-associated diseases lies in the different virulence factors possessed by different strains among which are the vacuolating cytotoxin A (*vacA*) and cytotoxin-associated gene A (*cagA*) [3,4].

Helicobacter pylori (*H. pylori*) infects more than half of the world's population [5]. Studies have shown that genes like the vacuolating cytotoxin (*vacA*), cytotoxin associated gene A (*cagA*), cytotoxin associated gene E (*cagE*) and blood adhesion binding antigen (*babA2*) may play important roles in the pathogenesis of *H. pylori* infection [6,7].

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The *cagA* gene is an indicator for the presence of the cag pathogenicity island (*cag* PAI) of about 40 kb and it is the first found to be present in *H. pylori* strains [8]. Its presence is associated with more severe clinical outcomes like peptic ulcer, atrophic G, and gastric cancer [9,3]. It induces interleukin-8 (IL-8) production and mucosal inflammation [9]. The *cag* PAI contains a gene (*cagE*) which is one of the marker genes in *cagI* of the *cag* PAI and it is required for translocation and phosphorylation [10,1I,12]. The *cagE* gene is associated with more severe clinical outcomes [13].

The *vacA* gene is found in all *H. pylori* strains and contains two variable parts, a signal region and a middle region allele [14]. Among the *vacA* subtypes, s1a, s1b, s1c and s2, and m1, m2a and m2b have been identified [15]. All strains of *H. pylori* have the *vacA* gene but they vary in their ability to produce cytotoxin [16]. Type m1 strains show more toxic activity than m2, type s1a is more active than s1b, and type s2 is less active than s1 [14].

Despite the association between *H. pylori* infection and gastrointestinal disorders, the specific genotypes of *H. pylori* strains in the Benue State region have not been comprehensively investigated. The heterogeneity in *vacA* and *cagA* genotypes among *H. pylori* strains contributes to variations in disease severity and clinical outcomes [14,17,18,19,20]. Understanding the prevalence and distribution of these virulence factors will help in tailoring diagnostic and therapeutic interventions to the population.

This research addresses this gap by focusing on the distribution of *vacA* and *cagA* genotypes among patients undergoing endoscopy at Benue State University Teaching Hospital in Makurdi, Nigeria with the aim of contributing not only to the academic understanding of microbial epidemiology but also to the practical improvement of clinical outcomes for patients in Benue State.

2. Materials and methods

2.1. Sample Collection

Eighty (80) gastric biopsy samples were obtained from the antrum of patients at Benue State University Teaching Hospital Makurdi during routine endoscopy procedures using sterile biopsy forceps. Multiple biopsy specimens were collected from different regions of the antrum and transported in Brain heart infusion broth to Safety Molecular and Pathology Laboratory, Enugu for genotypic analysis. Approval was obtained from the Medical Ethics Committee of the hospital and an informed consent was obtained from all patients.

2.2. DNA Extraction

Genomic DNA was extracted using a commercially available DNA extraction kit (ReliaPrep gDNA miniprep kit (Promega, Southampton, UK) and The extracted DNA was stored at -20 ^{o}C until used as template in PCR.

Specific primers for *vacA* and *cagA* genes were used based on already validated protocols [12]. PCR products were analyzed using gel electrophoresis

2.3. Singleplex PCR for Detection of *cagA* Region Gene of *H. pylori*

A singleplex PCR system was used to amplify the two regions of the *cagA* gene: 394bp and 717bp respectively.

The primer sequences used were:

CAGA394F	GATAACAGGCAAGCTTTTGAGGGA
CAGA394R	CCATGAATTTTTGATCCGTTC
CAGA717F	ATGGGGAGTCATGATGGCATAGAACC
CAGA717R[21].	ATTAGGCAAATTAAAGACAGCCACC

Water was used as no template control (NTC), *E. coli* DNA as Negative control (NC) and *H. pylori* samples as positive control (PC).

Exactly12.5µl of 10 x PCR master mix (or multiplex mix), 7.5µl of the CAG mix and 5.0µl of genomic DNA was pipetted into each sample well making a total reaction volume of 25µl.

The thermal profile set in the Eppendorf PCR machine as *cag* M mix was as follows: 95 °C for 3min, 94 °C for 60 sec, 58 °C for 60 sec, and 72 °C for 60 sec, 72 °C for 5 min for 40 cycles.

Electrophoresis was run in 2.0% agarose gel with 20µl ethidium bromide placed in 0.5 x TBE buffer at 100V for 45 minutes and viewed in ultra violet (UV) light. Product sizes 394bp or 717bp were considered *cagA* gene positive.

2.4. Multiplex PCR for Detection of *vacA* Mid Region (m) Gene of *H. pylori*

A single multiplex PCR system is used to amplify the mid region of the *vacA* gene, to identify the m1, m2, m1/m2 or m2/m1 alleles (strains).

The primer sequences used were:

VA7-F	GTAATGGTGGTTTCAACACC
VA7-R	TAATGAGATCTTGAGCGCT
VA4-F	GGAGCCCCAGGAAACATTG
VA4-R	CATAACTAGCGCCTTGCAC [9].

Water was used as no template control (NTC), *E. coli* DNA as Negative control (NC) and *H. pylori* samples as positive control (PC). Twelve point five microliters (12.5µl) of 10 x PCR master mix (or multiplex mix), 7.5µl of the *vac* m mix and 5.0µl of genomic DNA was pipetted making a total reaction volume of 25µl and put in each sample well. The thermal profile was set in the Eppendorf machine as *vac* m mix as follows: 95 °C for 3 min, 95 °C for 30sec, 56 °C for 60sec, 72 °C for 90sec, and 72 °C for 5min for 35 cycles. Electrophoresis was run in 2.0% agarose gel with 20µl ethidium bromide placed in 0.5 x TBE buffer at 100V for 45 minutes and viewed under UV light. The following product sizes were considered positive: 630bp for m1 alleles, 352bp for m2 alleles, 705bp for m1/m2 alleles and 277bp for m2/m1 alleles.

2.5. Multiplex PCR for Detection of vacA Signal Region(s) Gene of H. pylori

Two different multiplex PCR mixes are used to type the signal coding region ‘s’ region into s1 and s2 (s1 is sub-typed to s1a, s1b and s1c). Product sizes include 190bp for s1a, 187bp for s1b, 199bp for s2; 286bp for s2 and 259bp for s1 respectively.

The primer sequences used were as suggested by [9] and are:

VA1-F	ATGGAAATACAACAAACACAC
VA1-R	CTGCTTGAATGCGCCAAAC
VA1-s2-F	ATGGAAATACAACAAACACAC
VA1-s2-R	CTGCTTGAATGCGCCAAAC
SS1-F	GTCAGCATCACACCGCAAC
SS3-F	AGCGCCATACCGCAAGAG
SS2-F	GCTAACACGCCAAATGATCC

Water was used as no template control (NTC), *E. coli* DNA as Negative

Control (NC) and *H. pylori* strain from ATCC number 43526 as positive control (PC). Twelve point five microliters (12.5µl) of 10x PCR master mix (or multiplex mix), 7.5µl of the *primer* mix and 5.0µl of genomic DNA was pipetted making a total reaction volume of 25µl and put in each sample well. The Thermal Profile was set in the Eppendorf Machine as ‘*H. pylori* mix’ as follows: 95 °C for 3 min, 95 °C for 15 sec, 52 °C for 60 sec, 72 °C for 60 sec, 72 °C for 5 min, for 35 cycles. Electrophoresis was run in 2.0% agarose gel (with 20µl ethidium bromide placed in 0.5 x TBE buffer) at 100V for 30 minutes and the bands viewed in UV light. The Platinium Multiplex PCR master mix (Invitrogen, UK) was used in all multiplex reactions while all the primers were HPLC grade, synthesized by Eurofins, Germany.

3. Results

The frequency of *H. pylori* alleles of signal and mid regions of *vacA* detected in biopsies is shown in Table 1 where s1 had the highest frequency of 24 (100%) followed by s1c 22 (92%) while the least were s2, s1+s2 and m1+m2 (frequency=1; 4%) each. s1a was not detected.

The distribution of *H. pylori* genotypes detected in biopsies are shown in Table 2 where s1+m2 had the highest frequency of 22 (92%) followed by s1c+m2 19 (79%). The least were s2+m1, s1c+m1+m2 and s1c+s2+m2 1 (4%) each. Seven (29%) of the positive cases were *cagA* positive, 71% of the *cagA* positive were s1c+m2

The distribution of of *vacA* s and m alleles and *cagA* status from *H. pylori* infected patients are shown in table 3. s2 and m1 all had the highest positives *cagA* (100 %) each and m2 had the lowest *cagA* positives (22.7%).

Table 4 presents the distribution of *vacA* genotypes and *cagA* status from *H. pylori* infected patients. The s1c+m1+m2 and s1+s2+m2 all had *cagA* (100%) each and s1c+m2 had the least (26.32%) frequency of *cagA*.

Table 1 Frequency of *Helicobacter pylori* Alleles of Signal and Mid Regions of *vacA* Detected in Biopsies

Alleles	**Number**	**%**
s alleles		
s1a	0	0
s1b	2	8
s1c	22	92
s1	24	100
s2	1	4
s1+s2	1	4
M alleles		
m1	2	8
m2	22	92
m1+m2	1	4

Table 2 Frequency of *Helicobacter pylori* Genotypes Detected in Biopsies

Genotypes	**Number**	**%**
vacA		
s1+m2	22	92
s1+m1	2	8
s2+m2	1	4
s2+m1	0	0
s1c+m2	19	79
s1c+m1	1	4
s1b+m2	2	8
s1c+m1+m2	1	4
s1c+s2+m2	1	4

cagA		
cagA Positive	7	29
cagA⁺+ s1c+m2	5	71

Table 3 Distribution of *vacA* s and m Alleles and *cagA* Status from *Helicobacter pylori* Infected Patients (n=24)

***Vac A* s and m alleles**	**Number (%)**	***CagA* Positive (%)**	***CagA* Negative (%)**
s1			
s1b	2(8.3)	1(50)	1(50)
s1c	21(87.5)	5(23.8)	16(76.2)
s2	1(4.2)	1(100)	0(0)
Total	24(100)		
M			
m1	2(8)	2(100)	0(0)
m2	22(92)	5(22.7)	17(77.3)
Total	24(100)		

Table 4 Distribution of *vacA* Genotypes and *cagA* Status from *Helicobaeter pylori* Infected Patients (n=24)

***VacA* genotypes**	**Number (%)**	***CagA* Positive (%)**	***CagA* Negative (%)**
s1b+m2	2(8.3)	1(50)	1(50)
s1c+m1	1(4.2)	1(100)	0(0)
s1c+m2	19(79.1)	5(26.32)	14(73.68)
s1c+m1+m2	1(4.2)	1(100)	0(0)
s1+s2+m2	1(4.2)	1(100)	0(0)
Total	24(100)		

4. Discussion

The most prevalent *H. pylori* genotype in the area of study was the s1+ m2. A similar genotype was found in Nigerian patients with duodenal ulcer and non-ulcer dyspepsia [22].

The s1c subtype of *H. pylori* appears to be the most prevalent in the study population. Van Doorn *et al*. (1998) reported a similar finding in East Asia where subtype s1c appears to be the major allele. Similarly in Cukurova, Turkey [23], s1c was the most prevalent allele, and it was found to be significantly higher in patients with gastritis and or gastric ulcers.

The most prevalent specific subtype was the s1c+m2. This finding, from available literature, provides for the first time the specific subtypes of the *vacA* gene of *H. pylori* in Nigerian patients. According to [24], this specific subtype has lower virulence than the s1+m1 types. The Nigerian patients are infected mostly by the less toxic genotype [22], and this result may explain the lack of association between duodenal ulcers and *H. pylori* infection in Nigerian patients. Although *H. pylori* infection is prevalent in Nigeria and most developing countries in general, they do not share the same cytotoxicity genotype. The low prevalence of very toxic genotypes (s1+m1) in Nigerian dyspeptic patients as reported in this study, may explain the low frequency of serious gastrointestinal disorders such as gastric lymphoma and duodenal ulcers often associated with *H. pylori*.

The prevalence of *vacA* genotypes vary widely worldwide. In Pakistan [25], the most prevalent genotype was s1b+m2, in Thai [16], Ethiopia and Afghanistan ([26, 27] and Germany [15], it was the s1+m1 genotype, whereas in Columbia [28], it was the s1b+m1 genotype.

It was found that the s1+m1 genotype was predominant in Japan and Korea while s1b+m1 genotype was predominant in Columbia [29]. In Ethiopia and Afghanistan, amongst dyspeptic patients, the most prevalent genotype is the s1+m1. In Thai and Germany, the most prevalent genotype is the s1+m1 [16, 15] respectively. However, in Turkey, a high prevalence of s1+m2 genotype in gastritis patients was reported [29] which were similar with the present study.

In Kuwait patients, the *vacA* s1 type was prevalent among African Arabs, while the s2 type was common in South Asian patients [30]. Although *H. pylori* infection is prevalent in Nigeria and most developing countries in general, they do not share the same cytotoxicity genotype. The low prevalence of very toxic genotypes (s1+m1) in Nigerian dyspeptic patients, as reported in this study, may explain the low frequency of serious gastrointestinal disorders such as gastric lymphoma and duodenal ulcers often associated with *H. pylori*. However the prevalence of *cagA* in chronic gastritis patients differed markedly from the prevalence reported by [22] in duodenal ulcer and non-ulcer dyspeptic Nigerian patients.

The low prevalence of *cagA* as found in the study is similar to those of some previous studies [31,25,32] in Jordanian, Pakistani and Isreali patients respectively. A higher prevalence was however reported of *cagA* positive in North-Eastern region of Mexico [33].

Our findings reveal a nuanced distribution of *vacA* and *cagA* genotypes among *H. pylori* positive individuals, adding granularity to the understanding of the molecular epidemiology of *H. pylori* in the Benue State University Teaching Hospital population. The prevalence of *vacA* genotypes, as the most dominant variant, demostrates the allelic diversity reported in diverse geographical regions [14,17]. This diversity may contribute to variations in disease severity and clinical outcomes.

Similarly, the prevalence of *cagA*-positive strains agree with previous studies highlighting the association between *cagA* and severe gastroduodenal diseases [14, 17]. This finding also agrees with reports from Western countries [6,34] but reports from East Asian countries however showed that *cagA* was present in more than 90% of cases [28].

The prevalence of *cagA*-positive strains may serve as a potential indicator of increased virulence and disease risk within this population.

5. Conclusion

This study gives insights into the genotypic diversity of *Helicobacter pylori* among patients at Benue State University Teaching Hospital Makurdi. The distribution of *vacA* and *cagA* genotypes, along with the identification of distinct subtypes offers a foundation for personalized approaches to patient care. Ultimately, these findings contribute to the ongoing efforts to understand and mitigate the impact of *H. pylori*-associated diseases in the Benue State region and beyond.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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