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# Prevalence of *Helicobacter pylori vacA, cagA* and *iceA* genotypes in Nigerian patients with duodenal ulcer disease

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**Distinct virulence factors of *Helicobacter pylori* have been associated with clinical outcome of the infection; however, considerable variations have been reported from different geographic regions. Data on genotypes of African *H. pylori* isolates are sparse. The aim of this study was to determine the prevalence of specific genotypes of *H. pylori* in Nigerian patients with duodenal ulcer and non-ulcer dyspepsia. *H. pylori* was cultured from endoscopic biopsies obtained from 41 Nigerian patients (19 with duodenal ulcer, 22 with non-ulcer dyspepsia). The *vacA* alleles, *cagA* and *iceA* genotypes were determined by PCR. The *vacA* s1,m1 and s1,m2 genotypes were found in 26.3% and 22.7%, and in 73.7% and 72.7% of *H. pylori* isolates from patients with duodenal ulcer and non-ulcer dyspepsia, respectively. The *iceA*1 genotype was present in 94.7% and 86.4% of isolates from duodenal ulcer and non-ulcer dyspepsia patients, respectively. *cagA*$^+$ infection was found predominantly (>90%) in Nigerian *H. pylori* isolates irrespective of the clinical diagnosis. In conclusion, *vacA* s1,m2, *iceA*1 and *cagA*$^+$ are common genotypes of *H. pylori* isolated from Nigerian patients. As in several other developing countries there seems to be no association between these genotypes and duodenal ulcer disease.**

## Introduction

*Helicobacter pylori* is a common infection world-wide, the aetiological agent of chronic active gastritis and the major cause of peptic ulcer disease and primary gastric lymphoma of MALT type [1, 2]. *H. pylori* has also been recognised as a risk factor for gastric adenocarcinoma [3]. The reasons for these different outcomes of *H. pylori* infection remain unclear. Several *H. pylori* genes that are related to the risk of disease have been proposed. The cytotoxin-associated gene (*cagA*) is a marker for a genomic pathogenicity island of *c*. 40 kb and its presence is thought to be associated with a more severe clinical outcome of the infection [4, 5]. *cagA* is thought to be closely related to the vacuolating cytotoxin, encoded by the *vacA* gene. The *vacA* gene is present in virtually all *H. pylori* strains and contains at least two variable regions, the signal and the middle regions [6]. Strains harbouring the *vacA* s1 genotype show a high cytotoxic activity and have been linked to severe clinical disease such as peptic ulcer disease [7]. Another recently described putative virulence factor is *iceA* (induced by contact with the epithelium), which exists in at least two allelic forms, *iceA*1 and *iceA*2 [8]. *iceA1* is upregulated upon contact of *H. pylori* with the gastric epithelium and has been also been suggested as a marker for peptic ulcer disease [9].

Subsequent studies have shown considerable inconsistencies of these associations, depending on the population or geographic origin of the isolates [10, 11]. There are few data available as regards the pattern of *H. pylori* genotypes in patients from African populations [12, 13]. Therefore, the aim of this study was to investigate the prevalence of the *vacA*, *cagA* and *iceA* genotypes of *H. pylori* isolates from Nigerian patients with duodenal ulcer or non-ulcer dyspepsia.

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## Materials and methods

### *Patients and H. pylori isolates*

A total of 41 *H. pylori* isolates was obtained from 19 patients with active duodenal ulcer disease, and from 22 patients with dyspeptic symptoms, who had no current evidence and no history of peptic ulcer. All biopsies for primary *H. pylori* culture were obtained from the gastric antrum. The biopsies were smeared immediately on to Columbia agar base medium containing Dent's supplement and laked horse blood 7%. The plates were incubated at 37°C for 3–7 days under micro-aerobic conditions. *H. pylori* was identified by typical Gram's stain and colony morphology, and by positive biochemical tests for urease, catalase and oxidase.

### *H. pylori genotyping*

Bacterial chromosomal DNA was extracted from freshly harvested *H. pylori* cultures with phenol/chloroform. For PCR analysis of the targeted genes 2 $\mu$l of DNA were added to 50 $\mu$l of reaction mixture containing 1 × PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM (each) deoxynucleotide (Gibco BRL, Germany) and 0.5 $\mu$M of respective oligonucleotide primers. AmpliTaq Gold (Perkin Elmer, Germany) 2.5 U was added to each tube and overlaid with mineral oil. PCR was performed with a thermal cycler (GeneAmp 2400; Perkin Elmer). The amplification cycles (*cagA*, *vacA* s1/s2 and *iceA*) consisted of an initial denaturation of target DNA at 94°C for 9 min and then denaturation at 94°C for 1 min, primer annealing at 60°C or 56°C (*vacA* m1, m2) for 1 min and extension at 72°C for 1 min (35 cycles). The final cycle included an extension step for 5 min. The primers used to amplify the targeted genes are summarised in Table 1 [6, 9, 14]. Negative controls were added to each PCR run including all reagents except template DNA. *H. pylori* ATCC 49503 was used as the positive control. PCR products were visualised by agarose 1.5% gel electrophoresis.

## Results

Fig. 1 shows the results of electrophoresis of PCR products. The predicted 259-bp PCR product for *vacA* s1

**Table 1.** PCR primers for amplification of *cagA, vacA* and *iceA* sequences

| Gene | Primer sequence (5′–3′) | PCR product (bp) | Reference no. |
|---|---|---|---|
| *cagA* | TTGACCAACAACCACAAACCGAAG<br>CTTCCCTTAATTGCGAGATTCC | 183 | 14 |
| *vacA* s1/s2 | GGTCAAAATGCGGTCATGG<br>CTGCTTGAATGCGCCAAAC | 259/286 | 6 |
| *vacA* m1 | GGTCAAAATGCGGTCATGG<br>CCATTGGTACCTGTAGAAAC | 290 | 6 |
| *vacA* m2 | GGAGCCCCAGGAAACATTG<br>CATAACTAGCGCCTTGCAC | 352 | 6 |
| *iceA*1 | GTTGGGTAAGCGTTACAGAATTT<br>CATTGTATATCCTATCATTAC | 567 | 9 |
| *iceA*2 | GTTGGGTATATCACAATTTAT<br>TTRCCCTATTTTCTAGTAGGT | 229 or 334 | 9 |

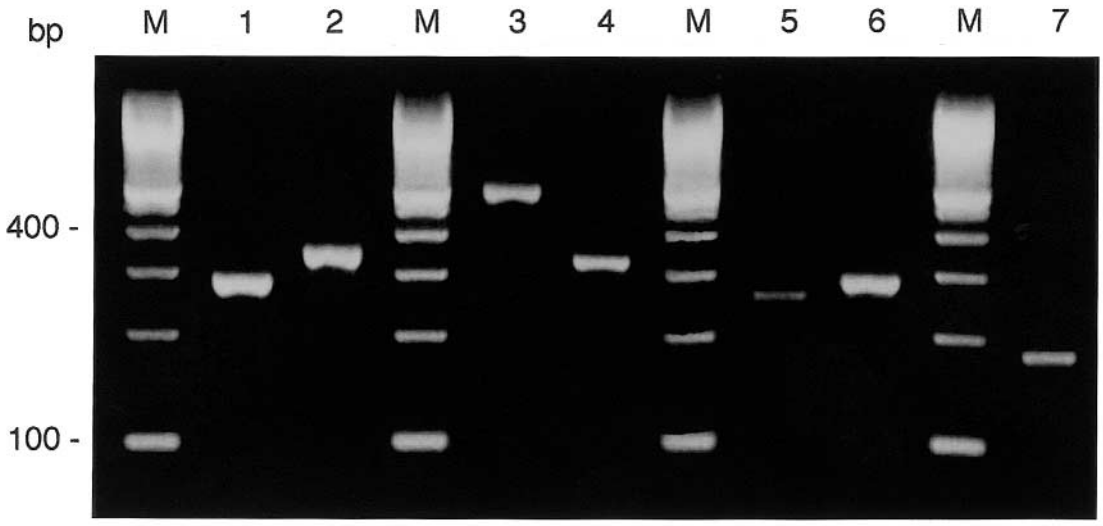


**Fig. 1.** Agarose 1.5% gel electrophoresis of PCR products for *H. pylori* genotyping. Lanes: **M**, 100-bp ladder; **1** and **2**, *vacA* m1 and m2; **3** and **4**, *iceA*1 and *iceA*2; **5** and **6**, *vacA* s1 and s2; **7**, *cagA*.

was identified in 40 *H. pylori* strains (98%). As shown in Table 2, the majority of isolates harboured the *vacA* s1,m2 genotype, followed by the s1,m1 genotype. A *vacA* s2,m1 genotype has not been found. There were no significant differences between the two patient groups.

The 183-bp PCR product indicating the presence of the *cagA* gene was obtained with 38 isolates (93%). *cagA*$^+$ infection was present in 95% of duodenal ulcer patients and in 91% of patients with non-ulcer dyspepsia ($p > 0.05$).

All *H. pylori* isolates possessed the *iceA* gene. In all, 37 isolates (90.2%) were positive for *iceA*1, of which 18 (94.7%) were from duodenal ulcer patients and 19 (86.4%) from non-ulcer dyspepsia patients (Table 2). A PCR product for both *iceA*1 and *iceA*2 was obtained from only one isolate from a non-ulcer dyspepsia patient.

The combined *vacA* s1/*cagA*$^+$/*iceA*1 genotype was present in 82.5% of all *H. pylori* isolates (Table 3).

## Discussion

The study demonstrated a high prevalence of *H. pylori* infection of the *vacA* s1, *cagA*$^+$/*iceA*1$^+$ genotype in patients from Nigeria with duodenal ulcer disease or non-ulcer dyspepsia. Therefore, this study is consistent with previous reports from the USA, Europe and Asia suggesting that *cagA* status and the *vacA* genotype of *H. pylori* may not predict the clinical outcome of infection [10, 15]. In contrast, another study from South Africa has shown that the *vacA* s1 allele accounted for 100% of ulcer strains and 67% of gastritis strains [12]. In that study, the *vacA* s2 type was found exclusively in gastritis patients, whereas in the present study the s2 allele was detected in only one patient with non-ulcer dyspepsia. In the present study, the *vacA* middle region types were not independently associated with the occurrence of peptic ulceration, which is in agreement with the study by Kidd *et al*. [12]. Another report by Letley *et al*. [13] supports these results, in that the *vacA* middle region polymorphism is not related to peptic ulceration. Kidd *et al*. also described the first case of a *vacA* s2,m1 *H. pylori* isolate from a duodenal ulcer patient [12]. The present study did not find such a genotype among a central African population. The prevalence of *cagA*$^+$ strains in Nigerian peptic ulcer patients and those with non-ulcer dyspepsia was similar to that shown in the study from South Africa. To our knowledge, this is the first study on alleles of the *iceA* gene in *H. pylori* isolates from Africa. In contrast to a previous report by van Doorn *et al*. [9], no association was found between the *iceA*1 allele and peptic ulcer disease. In another study by Yamaoka *et al*. [10], the *iceA*1 allele was predominant in Japanese and Korean populations, whereas *iceA*2 was prevalent in the USA and Colombia. An association between *iceA*1 and severity of clinical disease could not be shown in either population. This is also consistent with other studies from Asia suggesting that *cagA*, *vacA* and the *iceA* genotype were not associated with peptic ulcer disease [16].

In conclusion, this study demonstrated that *vacA* s1, *iceA*1 and *cagA*$^+$ are common genotypes of *H. pylori* in Nigeria. As in other developing countries, there seems to be no association between these genotypes and duodenal ulcer disease in this particular population.

**Table 2.** Genotype status of 41 *H. pylori* isolates from Nigerian patients with duodenal ulcer disease or non-ulcer dyspepsia

| Genotype | Number (%) of isolates from patients with duodenal ulcer disease (n = 19) | Number (%) of isolates from patients with non-ulcer dyspepsia (n = 22) | Total (%) |
|---|---|---|---|
| *vacA* s1m1 | 5 (26.3) | 5 (22.7) | 10 (24.4) |
| s1m2 | 14 (73.7) | 16 (72.7) | 30 (73.2) |
| s2m2 | 1 (4.5) | 1 (2.5) | 2 (4.9) |
| *cagA*$^+$ | 18 (94.7) | 20 (90.9) | 38 (92.7) |
| *iceA*1 | 18 (94.7) | 19 (86.4) | 37 (90.2) |
| *iceA*2 | 1 (5.3) | 2 (9.1) | 3 (7.3) |
| *iceA*1$^+$, *iceA*2$^+$ | 0 | 1 (4.5) | 1 (2.5) |

**Table 3.** Combined *vacA, cagA* and *iceA* genotypes

| Genotype | Number (%) of isolates from patients with Duodenal ulcer disease (n = 19) | Number (%) of isolates from patients with Non-ulcer dyspepsia (n = 21) | Total (%) |
|---|---|---|---|
| *vacA* s1/*cagA*$^+$/*iceA*1 | 17 (89.5) | 16 (76.2) | 33 (82.5) |
| *vacA* s1/*cagA*$^+$/*iceA*2 | 1 (5.3) | 2 (9.5) | 3 (7.5) |
| *vacA* s1/*cagA*$^-$/*iceA*1 | 1 (5.3) | 2 (9.5) | 3 (7.5) |
| *vacA* s2/*cagA*$^+$/*iceA*1 | 0 | 1 (4.8) | 1 (2.5) |

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