cagA and vacA Helicobacter pylori Pathogenicity Factors in Brazzaville, Congo

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Abstract

Introduction: CagA and VacA are the most important and well-studied virulence factors found in Helicobacter pylori. The aim of this work was to identify genes corresponding to H. pylori pathogenicity factors in Brazzaville, Congo. Material & Methods: A cross-sectional study was carried out from October 2013 to December 2016. Biopsy specimens were obtained from patients scheduled for upper gastrointestinal endoscopy in Brazzaville, Congo and were sent to the French National Reference Center for Campylobacters and Helicobacters in Bordeaux, France. H. pylori detection was conducted by real-time PCR using a fluorescence resonance energy transfer-melting curve analysis protocol. The identification of the genes encoding pathogenicity factors was carried out by conventional PCR using the appropriate primers for determination of CagA phosphorylation motifs 1, 2, 3; and vacAs, I and m regions: vacAi1, vacAi2, vacAs1a, vacAs1b. Results: A high prevalence of H. pylori infection was reported (108/143; 75.5%). In 92.2% (n = 71/77), the presence of P1, P2 and P3 CagA phosphorylation motifs was noted. Concerning vacA, vacAs1m1 was observed in 82% of the strains (n = 59/72). VacA1a was present in all strains (n = 76). With regard to the distribution according to the vacAs1 subtype, the majority of the strains (59/71; 83%) were vacAs1b positive, as compared to vacAs1c (17/34, 33%). The vacAs1a gene was absent in all of these patients. Conclusion: The presence of genes associated with severe gastric diseases indicates the importance of H. pylori eradication in the prevention of these diseases in Congo.
Keywords
Pathogenicity Factors, CagA, VacA

1. Introduction

More than 50% of the world population is infected with *Helicobacter pylori*. The bacterium is highly linked to peptic ulcer diseases (PUD). At least 10% of infected individuals develop PUD, and 1% - 3% develop gastric cancer [1]. The gastric cancer risk in *H. pylori* infected people is 2 to 7 times that of the uninfected. Ninety percent of distal gastric cancers are now considered to be the consequence of *H. pylori* infection. The WHO classified *H. pylori* as a group I carcinogen in 1994 (ref IARC) which they reconfirmed later [2].

*H. pylori* exerts its pathogenicity through/via several virulence factors, some of which influence colonization and the severity of the disease. One of the best characterized virulence factors is the CagA protein encoded by the cagA gene present in the *cag* pathogenicity island (cagPAI). The cagPAI also encodes a type IV secretion system (T4SS), representing a needle-like pilus, which is induced upon contact with host cells [3]. CagA is translocated by this T4SS across both the bacterial and host cell membranes into the cytoplasm of target cells. CagA represents a prime example of a tyrosine-phosphorylatable bacterial virulence factor [4] [5]. Upon delivery, members of the c-Src [3] and c-Abl host tyrosine kinase families were identified as having phosphorylated CagA. Mass spectrometry and site-directed mutagenesis of CagA identified a set of Glu-Pro-Ile-Tyr-Ala (EPIYA) repeat motifs as phosphorylation sites [5] [6].

CagA positive *H. pylori* strains are associated with increased inflammation and increased risk of PUD and gastric carcinoma in humans and experimental animals [7]. The presence of the protein CagA generally coincides with the presence of other virulence factors, including VacA, BabA and OipA [8]. Thus, the pathogenesis of *H. pylori* is multifactorial and cannot be reduced to a gene. The CagA protein is responsible for alterations of many cell signaling systems which profoundly influence the physiology of the host cell. When *H. pylori* is in contact with the host cells, the CagA protein is directly injected into the cytoplasm of the host cell where it is phosphorylated and binds to the host’s SHP2 domain [9]. SHP-2 is a phosphatase involved in signal transduction for the tyrosine kinase receptor [10]. CagA also causes the passage/transformation/evolution of an epithelial cell to a mesenchymal cell phenotype [11]. All of these phenotypes are associated with gastric carcinogenesis [12]. The vacuolating toxin VacA has been named for its ability to induce many large vacuoles in cultured cells. Unlike CagA, the VacA protein forms an autotransporter structure and secretes itself without the need for contact with the host cell. VacA proteins then oligomerize to form pore-like structures. VacA is transported to the receptor tyrosine phosphatase (RPTPα and RPTPβ) and other transmembrane glycosylated pro-
teins on the surface of the host cell [13]. VacA then enters by endocytosis and forms selective anion channels in the vacuole membrane. The channels allow the accumulation of chloride anions and weak bases, resulting in osmotic swelling [14]. VacA also inserts into mitochondrial membranes, causing mitochondrial dysfunction and apoptotic cell death [15]. Vacuolization is not the only effect of VacA intoxication. VacA disrupts the barrier function of epithelial cells, allowing leakage of essential nutrients such as iron, nickel, and amino acids. All *H. pylori* strains contain *vacA* genes, but not all strains produce a functional VacA protein. This is due to polymorphisms in the *vacA* gene, particularly at the amino-terminal (s region), in the middle of the gene (m region), and in the intermediate region (region i). The s2 polymorphism gives/results in an inactive toxin [16]. Thus, strains with the s2 allele are often called “VacA negative”. Polymorphisms have been discovered more recently and influence vacuolating activity; *vacA* containing the allele i1 produces the most active toxin. Strains harboring the s1m1 allele have been most commonly associated with PUD and gastric carcinomas, but it now appears that the i1 allele is more strongly associated with these diseases than the presence of the s1m1 genotype [16].

The aim of this work was to identify *cagA* and *vacA* polymorphism genotypes corresponding to *H. pylori* pathogenicity factors in Brazzaville, Congo.

2. Material and Methods

2.1. Obtention of Gastric Biopsies

A cross-sectional study was carried out between 2013 to December 2016.

Inclusion criteria: Biopsy specimens were obtained in the Schnell Clinic (a private medical clinic in Brazzaville, Congo), from patients who were never treated for *H. pylori* eradication, scheduled for an upper gastrointestinal endoscopy. Patients were aged 17 years and over and of any sex and consent to the study protocol. It was patients in routine consultation who consent to the study protocol.

The exclusion criteria were: the impossibility to perform a biopsy, incomplete endoscopy, a technical defect, a contra indication to perform a biopsy (the taking in the previous month of a treatment with anti-secretory gastric, antibiotic or anti-inflammatory no steroids).

2.2. *H. Pylori* DNA Extraction

Gastric biopsies were obtained and sent to the National Reference Center for Campylobacters and Helicobacters in Bordeaux, France where they were ground in 1 mL of brucella broth for molecular study. A small fragment was digested in 20 µl of proteinase K (Qiagen SA, Courtaboeuf, France) with 180 µl of lysis buffer (Qiagen) in 1.5 µl tube. This last tube was then placed on a block heating at 56°C at 1000 tours/minute and incubated overnight. DNA extraction was performed by using a MagNA Pure LC DNA isolation kit I (Qiagen), and then used to detect *H. pylori* and in the determination of the pathogenicity factors.
2.3. Detection of H. Pylori

Detection of *H. pylori* was performed by real-time PCR, which also determined point mutations in the 23S rRNA gene associated with clarithromycin resistance, as previously described [17]. The method included amplification of a fragment of the *H. pylori* 23S rRNA gene coupled with a simultaneous detection of the amplicon by probe hybridization, followed by a melting curve analysis [18].

2.4. CagPAI Empty Site PCR

cagPAI status was evaluated by amplification of cagA locus using conventional PCR, with previously described primers [19] [20].

Thus, specific primers for the cag empty site were also used to confirm the presence or absence of the cagPAI locus [21] ([22] Kersulyte et al., 1999).

The primers used are presented in Table 1.

The PCR were carried out in a 25 µl volume containing: 15 µl of water; 5 µl of PCR buffer 5× (Promega); 0.25 µl of a 10mM mixture of deoxynucleoside triphosphates (dNTPs) (Eurobio); 0.25 µl of *Taq* DNA polymerase (5 U/ml) (Eurobio); 1 µl of each primer (10 µM) and 2.5 µl of *H. pylori* DNA. After 2 minutes of denaturation at 95˚C, each reaction mixture was amplified for 40 cycles as follows: 30 sec at 95°C; 30 sec of annealing at 58˚C (for *cagA*); and 30 sec at 72˚C. After the last cycle, extension was continued for another 5 min at 72˚C. All PCR products were analyzed on a 2% agarose gel stained with ethidium bromide. When the cagPAI gene was absent, a 324 bp band was observed. *H. pylori* DNA extracts from strains GC 34 and 3829 were used as controls.

2.5. CagA Phosphorylation Motifs Detected by PCR

CagA phosphorylation motifs were determined by conventional PCR. The primers used are presented in Table 1.

The PCR was carried out in a 25 µl volume containing: 15.875 µl of water; 5 µl of PCR buffer 5× (Promega); 0.5 µl of 10 mM of a mixture of deoxynucleoside triphosphates (dNTPs) (Eurobio); 0.25 µl of *Taq* DNA polymerase (5 U/ml) (Eurobio); 1 µl of each primer (10 µM) and 2.5 µl of *H. pylori* DNA. After 2 minutes of denaturation at 95˚C, each reaction mixture was amplified for 40 cycles as follows: 30 sec at 95°C; 30 sec of annealing at 58˚C (for *cagA*); and 30 sec at 72˚C. After the last cycle, extension was continued for another 5 min at 72˚C. All PCR products were analyzed on a 2% agarose gel stained with ethidium bromide. When the cagPAI gene was absent, a 324 bp band was observed. *H. pylori* DNA extracts from strains GC 34 and 3829 were used as controls.

### Table 1. Primers for genotyping of CagA.

| Primers   | Sequence (5’-3’) | Reference                  |
|-----------|------------------|---------------------------|
| 468 HP519 | GCT TGC TTG TAT TGG CCT TG GCA TGC ACA TTC CCT AAA GTG | Achtman et al., 1999; Kersulyte et al., 1999 |
| 496 HP549 | GCT TGC TTG TAT TGG CCT TG GCA TGC ACA TTC CCT AAA GTG | Kersulyte et al., 1999 |
| cagA-P1C  | TTCTCAAGGAGCAATTGGC | Argent et al., 2005 |
| cagA-P2CG | TTAGCAACTTGAGCGTAAATGGG | Argent et al., 2005 |
| cagA-P2TA | TTAGCAACTTGAGTATAAATGGG | Argent et al., 2005 |
| cagA-P3E  | ATCAATGTAGCGTAAATGGG | Argent et al., 2005 |
| cagA-pD   | TTGATTGGCCTCATCAAAATC | Jones et al., 2009 |
triphosphates (dNTPs) (Eurobio); 0.125 µl of *Tag* DNA polymerase (5 U/ml) (Eurobio); 0.5 µl of each primer (25 µM) and 2.5 µl of *H. pylori* DNA. After 2 min of denaturation at 95°C, each reaction mixture was amplified for 35 cycles (for *cagA* phosphorylation motifs genes P1 and P2) and 45 cycles (for *cagA* phosphorylation motif P3) as follows: 30 sec at 95°C; 30 sec of annealing at 57°C (for *cagA*); and 20 sec at 72°C (*CagA* P1) or 25 sec at 72°C (*cagA* P2) or 50 sec at 72°C (*cagA* P3). After the last cycle, extension was continued for another 5 minutes at 72°C. All PCR products were analyzed on a 2% agarose gel stained with ethidium bromide. A264 bp band was observed for cagAP1, 309 pb for cagAP2, and 485 pb for cagA P3. Reference DNA extracts from *H. pylori* strains J99 and 7.13 were used as controls.

2.6. *VacA* Genotyping PCR

The *vacA* signal (s) and middle (m) regions were typed by conventional PCR, using the primers as previously described (Table 2) [23] [24]. The patients were identified at first as type s1 or s2 and type m1 or m2. All extract DNA with signal region type s1 were further characterized into s1a, s1b or s1c variants by performing three separate PCR assays. Thermal cycling conditions for each set of primers (0.5 µM) were 95°C for 1 min, and 52°C for 1 min, for a total of 35 cycles. After 2 min of denaturation at 94°C, each reaction mixture was amplified for 35 cycles as follows: 30 sec at 94°C; 30 sec of annealing at 58°C (for *vacAi1* and *vacAi2*) or 30 sec of annealing at 60°C (*vacA s, m, s1a, s1b and s1c); 30 sec at 72°C (*vacA s, m, s1a, s1b and s1c) or 40 sec at 72°C (for *vacAi1* and *vacAi2*).

After the last cycle, extension was continued for another 5 min at 72°C. All PCR products were analyzed on a 2% agarose gel stained with ethidium bromide. A 567 bp band was observed for vacAm1, a 642 bp band for vacAm2, a 259 bp band for vacAs1 and a 286 bp band for vacAs2. DNA extracts from *H. pylori* strains J99 ss1, 7.13, B38, 26695 were used as controls.

2.7. Statistical Analysis

The data were analyzed using the GraphPad Prism 7 software. The chi-square test ($\chi^2$) was used to compare the genotype frequencies of *cagA* and *vacA* and the frequencies of upper gastrointestinal endoscopy results. The confidence

| Table 2. Primers for genotyping of *vacA* s and m. |
|---------|---------|---------|
| Primers | Sequence (5’-3’) | Reference |
| VA1-F   | ATGGAAATACAAACAACACAC | Atherton *et al.*, 1995 |
| VA1-R   | CTGCTTGAATGCGCCAACAC |  |
| VAG-F   | CAATCTGCTCAAATCAGCGAG |  |
| VAG-R   | GCGTCAAATAATTCAGG |  |
| C1R     | TTAATTTTAACGCTGTITAAG | Rhead *et al.*, 2007 |
| C2R     | GATCAACGCTCTGATTG |  |
interval was 95%. The difference between the frequencies was considered significant when the $p$-value was less than 0.05.

3. Results

3.1. Characteristics of the Patients

A total of 143 patients were included in the study. Seventy-one patients (49.7%) were male and 72 (50.3%) female (sex ratio F/M = 1); 120 patients (83.9%) were outpatients and 23 (16.1%) were hospitalized. The age of the patients was between 17 and 76 years, with an average mean age of 43.9 $\pm$ 15.3 years.

3.2. H. pylori Prevalence

A high prevalence of $H.\ pylori$ infection was reported (108/143; 75.5%). The prevalence in the 17 - 37 year age group was 95.8% (46+/48), in the 38 - 58 year age group 85.1% (46+/54), and in the 59 - 76 year age group 83.3% (35+/41) ($p > 0.05$).

3.3. Distribution of vacA and cagA Alleles

The $cagPAI$ was present in 93.9% (77/82) and absent in 6.1% (5/82). Then, the prevalence of $cagA$ genotype was noted in 93.9% (77/82). In 92.2%, the presence of P1, P2 and P3 phosphorylation motifs of the $cagA$ were noted and vacA s1m1 was present in 82%. The prevalence of the different pathogenicity factors and their relationship with the upper gastrointestinal endoscopy results are presented in Table 3 and Table 4. Figures 1-3 present the 2% Agarose gel

| Pathogenicity factors (genes) | n   | %   |
|------------------------------|-----|-----|
| $cagA$ allele (N = 77)        |     |     |
| $cagA$P1, P2, P3             | 71  | 92.2|
| $cagA$P2, P3                 | 3   | 3.9 |
| $cagA$P3                     | 3   | 3.9 |
| vacA alleles s and m (N = 72) |     |     |
| vacAs1m1                     | 59  | 81.9|
| vacAs2m1                     | 1   | 1.4 |
| vacAs2m2                     | 1   | 1.4 |
| vacAs1                      | 9   | 12.5|
| vacAs2                      | 1   | 1.4 |
| vacAm1                      | 1   | 1.4 |
| vacA allele i1 (N = 76)      |     |     |
| vacA allele s1a (N = 71)     |     |     |
| vacA allele s1b (N = 71)     | 59  | 83  |
| vacA allele s1c (N = 51)     | 17  | 33.3|
Figure 1. 2% Agarose gel electrophoresis of PCR amplicon of the phosphorylation motif CagA 3 (P3); M = DNA lab marker; CN = negative control; J99 and 7.13: positive control; 1, 2, 3 and 4: fragments of positive cagA *H. pylori* genotypes.

Figure 2. 2% Agarose gel electrophoresis of PCR amplicon of the VacA m gene; M = DNA lab marker; 2, 4 and 6: fragments of positive VacA m1 and m2 *H. pylori* genotypes.

Figure 3. 2% Agarose gel electrophoresis of PCR amplicon of the VacA s gene; M = DNA lab marker; 2, 4 and 6: fragments of positive VacA s1 and s2 *H. pylori* genotypes.
Table 4. cagA and vacA genotypes and upper gastrointestinal endoscopy results.

| Gene characteristic | cagA and vacA genotypes and upper gastrointestinal endoscopy results | Gastritis | No gastritis | P value |
|---------------------|-------------------------------------------------|-----------|-------------|---------|
|                     |                                                 | n         | %           | n       | %       |         |
| cagA (N = 82)       |                                                 | 47        | 61.04       | 30      | 38.96   | 0.9999  |
| Present (N = 77)    |                                                 | 3         | 60          | 2       | 40      |         |
| Absent (N = 5)      |                                                 | 20        | 33.9        | 39      | 66.1    |         |
| vacAs1m1 (N = 59)   |                                                 | 8         | 61.54       | 5       | 38.46   | 0.0642  |
| vacA s et m nons1m1 (N = 13) |                           | 31        | 52.54       | 28      | 47.46   |         |
| vacAs1b (N = 59)    |                                                 | 7         | 58.33       | 5       | 41.67   | 0.3666  |
| vacA s nons1b (N = 12) |                               | 9         | 52.95       | 8       | 47.05   |         |
| vacA s1c (N = 17)   |                                                 | 21        | 61.76       | 13      | 38.24   | 0.3461  |
| vacA nons1c (N = 34) |                               | 21        | 61.76       | 13      | 38.24   |         |

Not statistically significant (p > 0.05).

electrophoresis of PCR amplicon of the phosphorylation motif Cag A 3 (P3), VacA m1 and m2 and VacA s1 and s2. There was no significant difference between the male and female patients with regard to the pathogenicity factors (p > 0.05).

4. Discussion

This study reports a high prevalence of H. pylori infected individuals in the Congo (75.5%). These results are similar to those in the study by Ankouane Andoulo et al. in Cameroun, who reported a prevalence of 72.5% [25].

Indeed, most patients harbour strains with the cagA gene (93.9%) and all cagA gene have phosphorylatable motifs with 92.2% of cagAP1, P2, P3. Some studies reported that cagA is present in approximately 70% of strains worldwide, but this rate varies geographically, from between 90% - 95% in East Asian countries (South Korea, China, Japan) to only about 40% in Western countries [26] [27]. In Africa, Kidd, Lastovica, Atherton, et al. found the presence of cagA in all South African strains [28]. Our results indicate that our patients, are at risk for severe gastroduodenal diseases due to the CagA proteins. Indeed, H pylori could directly deliver the CagA protein into the host epithelial cell cytoplasm via the cagPAI-coded type IV export system [29]. Inside the epithelial cells, the CagA protein undergoes tyrosine phosphorylation by the host Src family protein tyrosine kinases, and the CagA protein binds an Src homology 2 (SH-2) domain-containing tyrosine phosphatase SHP-2, and stimulates the division and proliferation of gastric epithelial cells [9]. The CagA-Csk interaction activates Csk and inactivates the Src family kinases, thereby bringing about a decrease in CagA tyrosine-phosphorylation as well as in CagA-SHP2 interactions as a feedback mechanism [30]. Through/Via this mechanism, chronic infection with
CagA-positive strains persist, thus causing the host damage. A typical characteristic of AGS gastric epithelial cells infected with cagPAI-positive H. pylori is their "hummingbird" phenotype [4] [6]. This in vitro phenotype likely mirrors numerous in vivo signaling activities that control host cell motility, invasive growth and metastasis of cancer cells [31] [32]. Otherwise the oncogenic role of CagA is further supported by in vivo experiments in mice, where transgenic cagA expression in the stomach leads to gastric epithelial hyperplasia, adenocarcinoma, myeloid leukemia and B-cell lymphoma [33] [34].

In addition, patients with chronic H. pylori infection in Brazzaville also have the risk of developing gastritis and ulcer pathologies. Indeed, this demonstrates that the CagA protein is a multiple effector via phosphorylation independent via T4SS to activate the NF-κB-inducing kinase (NIK) and IkB kinase αβ (IKKαβ) resulting in subunit IkBa of NF-κB (trimer IkBa/p50/p60) phosphorylation and then degradation [35]. Active NF-κB (dimer p50/p60) translocates into the nucleus to transcribe the inflammatory factor genes [cyclooxygenase-2 (COX-2), intercellular adhesion molecule-1 (ICAM-1), and inducible nitric oxide synthase (iNOS)], proinflammatory cytokine genes [interleukin-6 (IL-6), interferon-γ (INF-γ), and tumor necrosis factor-α (TNF-α)], and the chemokine IL-8 gene [35]. This is called the NF-κB pathway. All of these related proteins can result in severe inflammation of the gastric mucosa for infected cells [26] [35] [36] [37] (Figure 4).

The vacA gene represents another locus involved in the disease. Concerning the vacA gene polymorphisms in this study, variations of the vacA alleles (s1a, s1b, s1c, or s2), (m1 or m2), or (i1 or i2) also exist. This study noted that 81.9% of patients have the allelic combination s1m1 and all of those tested (100%) have the i1 allele. H. pylori strains with vacA alleles s1/m1/i1 are associated with an increased risk of developing severe disease, compared to positive s2/m2/i2 vacA strains [24] [38]. In fact, among the possible allelic combinations, the vacAs1/m1 alleles are the most virulent combination, while the s1/m2 and s2/m2 genotypes display virtually no cytotoxicity [39].

Together, while each of the vacA polymorphisms has been used as a predictor for VacA-induced disease severity, it is clear that other factors, including the presence of CagA (discussed below), contribute to disease. For this reason, individually typing vacA alone may not provide sufficient information to understand the virulence potential of a strain and multiple virulence factor typing appears to be required to understand strain dependent disease contributions [39].

As shown in Figure 5, VacA oligomer p88 forms anionselective channels in the cytoplasmic membrane, which can further react with early and late endosomal compartments (EE/LE) to form anion-selective channels in the vacuole membrane. Such channels increase permeability to small organic molecules and cations Fe³⁺/Ni²⁺ which can further interact with NH₄⁺ from H. pylori generating an osmotic force for the driving water influx and vesicle swelling, finally leading to vacuolation [26] [35]. On the other hand, the p88/EE/LE complex could be activated by Bax and Bak, resulting in mitochondrial transmembrane
Figure 4. CagA and known host cell targets [26]. (a) A schematic representation of CagA with the polymorphic region containing different EPIYA motif (A, B, C, and D) combinations is shown and was adapted from that of Hatakeyama and Higashi (2005); (b) A graphic depiction of the gastric mucosa and known host pathways impacted by phosphorylated and non-phosphorylated CagA is shown. Pathways targeted in epithelial cells and B cells are indicated. The actin binding proteins (ABP) affected by CagA include vinculin, cortactin, and ezrin. This figure was adapted from an earlier version by Rieder et al. (2005).

potential (ΔΨm) disruption, followed by the release of cytochrome c from mitochondria to cytoplasm, activation of caspase-9 and caspase-3, and finally proceeding to apoptosis. However, apoptosis is inhibited by CagA [26] [35].

In our study, there was no statistically significant difference regarding the cagA gene or vacA alleles and age group, gastritis and another pathology in the study population. These results are on the contrary of those obtained in some studies. El Khadir noted a difference in the vacA and cagA combination of H. pylori in PUD and gastric cancer cases [20] [40]. Lehours did not find a difference between gastritis and gastric MALT lymphoma patients regarding H. pylori
Figure 5. VacA and known host cell targets [26]. (a) A schematic representation of VacA with the three major regions of polymorphisms (s, i, and m) is shown. Additionally, known alleles of corresponding to each region are shown. The i region contains two important polymorphic regions known as Cluster B and Cluster C, which are designated by B and C, respectively, on the diagram. The activity attributed to each of the regions of the toxin (vacuolating activity or cellular tropism) are indicated, and the impact of each allele on these effects is shown. The highest level of activity or the broadest tropism is defined as ++, intermediate tropism is indicated by a +, low activity is indicated as a +/-, no activity is designated by a −, and incomplete information is indicated by a ?; (b) A depiction of the gastric mucosa and known host pathways targeted by VacA is shown. One of the receptors, sphingomyelin is designated by SM. Pathways targeted in epithelial cells and B and T cells are indicated. Additionally, activation of several pathways by peptidoglycan (PG) and LPS are shown. This figure was adapted from an earlier version by Rieder et al. (2005).

Our results can be explained by the fact that H. pylori is acquired early in childhood, as there is no statistically significant difference between the age groups and the strains exist in many parts of the population, even if the results of the endoscopy are normal [41].

The results of this study can explain the frequency of gastritis, PUD and gastric cancers in Brazzaville, Congo. Indeed, Ibara et al. reported 62.02% gastritis (first cause of gastric pathologies), 11.29% PUD and 3.60% gastric adenocarcinomas, despite the fact that a causal relationship with H. pylori has not been es-
H. pylori infection and gastric carcinogenesis processes in Congo must be fought using strategies based on the recommendation for H. pylori diagnosis and antimicrobial susceptibility testing in order to eradicate H. pylori and prevent gastritis, PUD and carcinoma.

Despite the evidence that cagA positivity, CagA and VacA seropositivity, and/or vacA polymorphism contribute to disease severity, numerous studies have not found this association [26] [43].

For example, in Tunisia, the vacA type is significantly different between patients with peptic ulceration and gastritis, while CagA status is not [26] [44].

In China, no association between cagA status and peptic ulceration or chronic gastritis was established, due likely to the high presence of CagA in both patient populations. The differences observed between disease severity and toxin type/presence in these epidemiological studies may be due to differences that exist in H. pylori strains well beyond the described cagA and vacA polymorphisms. As noted at the outset of this review, environmental, geographic, and host influences could contribute to the differences observed in disease severity between these studies. As such, while individual evaluation of cagA and vacA genotypes show that both contribute to disease, the lack of evaluation of both genotypes in combination with other factors is problematic in determining how both toxins contribute to disease [26].

5. Conclusion

The presence of genes associated with severe gastric diseases indicates the importance of H. pylori eradication in the prevention of these diseases in Congo.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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E. N. Ontsira Ngoyi et al.

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