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cagA and vacA Helicobacter pylori Pathogenicity Factors in Brazzaville, Congo

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Abstract

Introduction: *Cag*A and *Vac*A 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 *Cag*A phosphorylation motifs 1, 2, 3; and *vac*As, I and m regions: *vac*Ai1, *vac*Ai2, *vac*As1a, *vac*As1b. **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 *Cag*A phosphorylation motifs was noted. Concerning *vac*A, *vac*As1m1 was observed in 82% of the strains (n = 59/72). *Vac*ai1 was present in all strains (n = 76). With regard to the distribution according to the *vac*As1 subtype, the majority of the strains (59/71; 83%) were *vac*As1b positive, as compared to *vac*As1c (17/34, 33%). The *vac*As1a 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, *Cag*A, *Vac*A

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 *Cag*A protein encoded by the *cag*A gene present in the *cag* pathogenicity island (*cag*PAI). The *cag*PAI also encodes a type IV secretion system (T4SS), representing a needle-like pilus, which is induced upon contact with host cells [3]. *Cag*A is translocated by this T4SS across both the bacterial and host cell membranes into the cytoplasm of target cells. *Cag*A 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 *Cag*A. Mass spectrometry and site-directed mutagenesis of *Cag*A identified a set of Glu-Pro-Ile-Tyr-Ala (EPIYA) repeat motifs as phosphorylation sites [5] [6].

*Cag*A 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 *Cag*A generally coincides with the presence of other virulence factors, including *Vac*A, BabA and OipA [8]. Thus, the pathogenesis of *H. pylori* is multifactorial and cannot be reduced to a gene. The *Cag*A 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 *Cag*A 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]. *Cag*A 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 *Vac*A has been named for its ability to induce many large vacuoles in cultured cells. Unlike *Cag*A, the *Vac*A protein forms an autotransporter structure and secretes itself without the need for contact with the host cell. *Vac*A proteins then oligomerize to form pore-like structures. *Vac*A is transported to the receptor tyrosine phosphatase (RPTPα and RPTPβ) and other transmembrane glycosylated pro-

teins on the surface of the host cell [13]. *Vac*A 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]. *Vac*A also inserts into mitochondrial membranes, causing mitochondrial dysfunction and apoptotic cell death [15]. Vacuolization is not the only effect of *Vac*A intoxication. *Vac*A disrupts the barrier function of epithelial cells, allowing leakage of essential nutrients such as iron, nickel, and amino acids. All *H. pylori* strains contain *vac*A genes, but not all strains produce a functional *Vac*A protein. This is due to polymorphisms in the *vac*A 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 "*Vac*A negative". Polymorphisms have been discovered more recently and influence vacuolating activity; *vac*A 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 *cag*A and *vac*A 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. *Cag*PAI Empty Site PCR

*cag*PAI 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 *cag*PAI 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 5X (Promega); 0.25 µl of a 10mMmixture 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 *cag*PAI gene was absent, a324 bp band was observed. *H. pylori* DNA extracts from strains GC 34 and 3829 were used as controls.

2.5. *Cag*A Phosphorylation Motifs Detected by PCR

*Cag*A 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

Table 1. Primers for genotyping of *CagA*.

Primers	Sequence (5'-3')	Reference
	CagPAI empty site	
468 HP519 496 HP549	GCT TGC TTG TAT TGG CCT TG GCA TGC ACA TTC CCT AAA GTG	Achtman et *al.*, 1999; Kersulyte et *al.*, 1999
	CagA phosphorylation motifs	
*cagA*28F	TTCTCAAAGGAGCAATTGGC	
cagA-P1C	GTCCTGCTTTCTTTTTATTAACTTK AGC	
cagA-P2CG	TTTAGCAACTTGAGCGTAAATGGG	Argent *et al.*, 2005
cagA-P2TA	TTTAGCAACTTGAGTATAAATGGG	
cagA-P3E	ATCAATTGTAGCGTAAATGGG	
cagA-pD	TTGATTTGCCTCATCAAAATC	Jones *et al.*, 2009

triphosphates (dNTPs) (Eurobio); 0.125 µl of *Taq* 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 motifP3) as follows: 30 sec at 95˚C; 30 sec of annealing at 57˚C (for *cagA*); and 20 sec at 72˚C (*Cag*A 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 *cag*AP1, 309 pb for *cag*AP2, and 485 pb for *cag*A 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 *vac*Ai1 *and vac*Ai2) or 30 sec of annealing at 60˚C (*vac*A s, m, s1a, s1b and s1c); 30 sec at 72˚C (*vac*A s, m, s1a, s1b and s1c) or 40 sec at 72˚C (for *vacA*i1 *and vacA*i2). 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 *vac*Am1, a642 bp band for *vac*Am2, a259 bp band for *vac*As1 and a286 bp bandfor *vac*As2. 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 (Ki^2) was used to compare the genotype frequencies of *cag*A and *vac*A and the frequencies of upper gastrointestinal endoscopy results. The confidence

Table 2. Primers for genotyping of v*acA s and m.*

Primers	Sequence (5'-3')	Reference
VA1-F	ATGGAAATACAACAAACACAC	Atherton *et al.*, 1995
VA1-R	CTGCTTGAATGCGCCAAAC	
VAG-F	CAATCTGTCCAATCAAGCGAG	
VAG-R	GCGTCAAAATAATTCCAAGG	
C1R	TTAATTTAACGCTGTTTGAAG	Rhead *et al.*, 2007
C2R	GATCAACGCTCTGATTTGA	

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 +/− 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 *vac*A and *cag*A Alleles

The *cag*PAI was present in 93.9% (77/82) and absent in 6.1% (5/82). Then, the prevalence of *cag*A genotype was noted in 93.9% (77/82). In 92.2%, the presence of P1, P2 and P3 phosphorylation motifs of the *cag*A were noted and *vac*A 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

Table 3. Prevalence of *cag*A and *vac*A genotypes.

Pathogenicity factors (genes)	Frequency	
	n	%
cagA allele (N = 77)		
*cag*AP1, P2, P3	71	92.2
*cag*AP2, P3	3	3.9
*cag*AP3	3	3.9
vacA alleles s and m (N = 72)		
*vac*As1m1	59	81.9
*vac*As2m1	1	1.4
*vac*As2m2	1	1.4
*vac*As1	9	12.5
*vac*As2	1	1.4
*vac*Am1	1	1.4
vacAallele i1 (N = 76)	76	100
vacAallele s1a (N = 71)	00	00
vacAallele s1b (N = 71)	59	83
vacAallele s1c (N = 51)	17	33.3

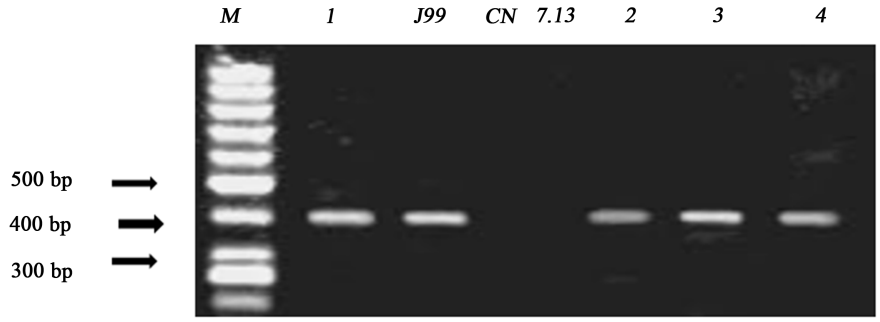

Figure 1. 2% Agarose gel electrophoresis of PCR amplicon of the phosphorylation motif *Cag*A 3 (P3); M = DNA lab marker; CN = negative control; J99 and 7.13: positive control; 1, 2, 3 and 4: fragments of positive cagA *H. pylori* genotypes.

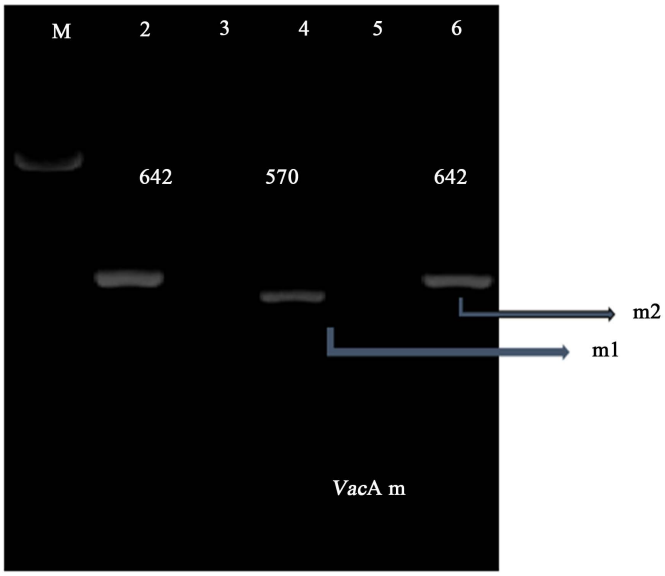

Figure 2. 2% Agarose gel electrophoresis of PCR amplicon of the *Vac*A m gene; M = DNA lab marker; 2, 4 and 6: fragments of positive *Vac*A m1 and m2 *H. pylori* genotypes.

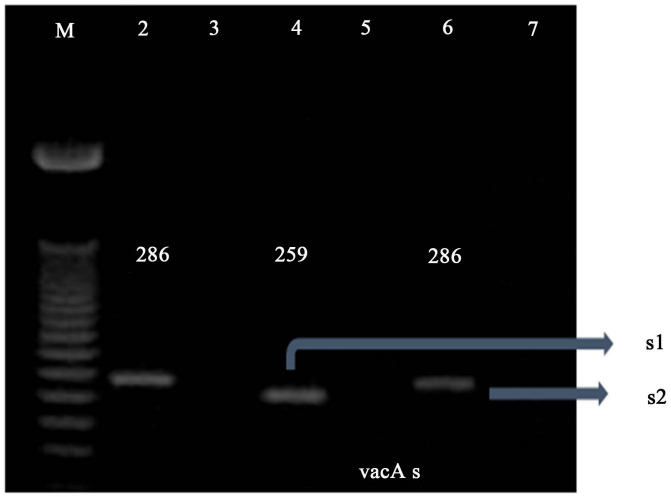

Figure 3. 2% Agarose gel electrophoresis of PCR amplicon of the *Vac*A s gene; M = DNA lab marker; 2, 4 and 6: fragments of positive *Vac*A s1 and s2 *H. pylori* genotypes.

Table 4. cagA and *vac*A genotypes andupper gastrointestinal endoscopy results.

Gene characteristic	*cag*A and *vac*A genotypes and upper gastrointestinal endoscopy results				P value
	Gastritis		No gastritis		
	n	%	n	%	
***cag*A (N = 82)**					
Present (N = 77)	47	61.04	30	38.96	0.9999
Absent (N = 5)	3	60	2	40	
***vacA*s1m1 (N = 59)**	20	33.9	39	66.1	0.0642
***vacA s et m* nons1m1 (N = 13)**	8	61.54	5	38.46	
***vac*As1b (N = 59)**	31	52.54	28	47.46	0.3666
***vac*A *s* nons1b (N = 12)**	7	58.33	5	41.67	
***vac*A s1c (N = 17)**	9	52.95	8	47.05	0.5461
***vac*A non s1c (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), *Vac*A m1 and m2 and *Vac*A 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 *cag*A gene (93.9%) and all *cag*A gene have phosphorylatable motifs with 92.2% of *cag*AP1, P2, P3. Some studies reported that *cag*A 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 *cag*A in all South African strains [28]. Our results indicate that our patients, are at risk for severe gastroduodenal diseases due to the *Cag*A proteins. Indeed, *H pylori* could directly deliver the *Cag*A protein into the host epithelial cell cytoplasm via the *cag*PAI-coded type IV export system [29]. Inside the epithelial cells, the *Cag*A protein undergoes tyrosine phosphorylation by the host Src family protein tyrosine kinases, and the *Cag*A 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 *Cag*A-Csk interaction activates Csk and inactivates the Src family kinases, thereby bringing about a decrease in *Cag*A tyrosine-phosphorylation as well as in *Cag*A-SHP2 interactions as a feedback mechanism [30]. Through/Via this mechanism, chronic infection with

*Cag*A-positive strains persists, thus causing the host damage. A typical characteristic of AGS gastric epithelial cells infected with *cag*PAI-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 *Cag*A 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 *Cag*A protein is a multiple effector *via* phosphorylation independ/via T4SS to activate the NF-κB-inducing kinase (NIK) and IκB kinase α/β (IKKα/β) resulting in subunit IκBα of NF-κB (trimer IκBα/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 *vac*A gene represents another locus involved in the disease. Concerning the *vac*A gene polymorphisms in this study, variations of the *vac*A 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 *vac*A alleles s1/m1/i1 are associated with an increased risk of developing severe disease, compared to positive s2/m2/i2 *vac*A strains [24] [38]. In fact, among the possible allelic combinations, the *vac*As1/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 *vac*A polymorphisms has been used as a predictor for *Vac*A-induced disease severity, it is clear that other factors, including the presence of *Cag*A (discussed below), contribute to disease. For this reason, individually typing *vac*A 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**, *Vac*A 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 Fe^{3+}/Ni^{2+} which can further interact with NH_4^+ 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

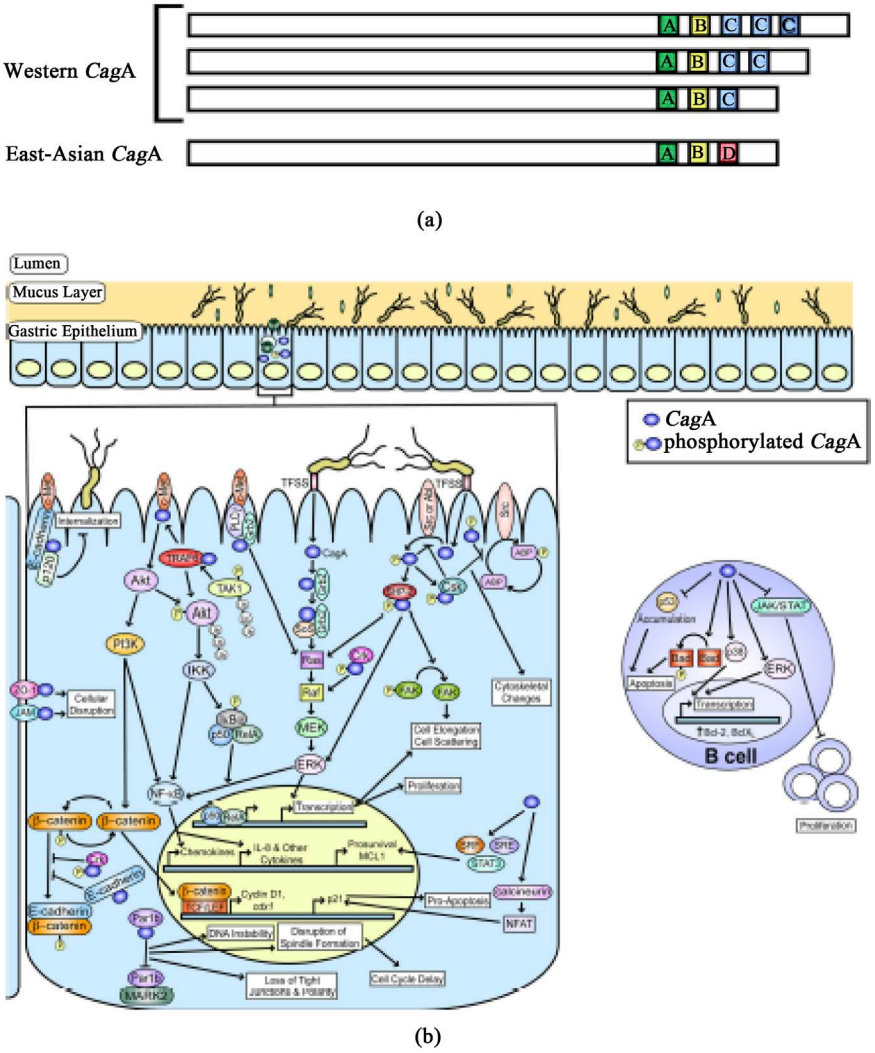

Figure 4. *Cag*A and known host cell targets [26]. (a) A schematic representation of *Cag*A with the polymorphic region containing different EPIYA motif (A, B, C, and D) combinations is shown and was adapted from that of Hatakeyama and Higashi (2005); (b) A graphic depiction of the gastric mucosa and known host pathways impacted by phosphorylated and non-phosphorylated *Cag*A is shown. Pathways targeted in epithelial cells and B cells are indicated. The actin binding proteins (ABP) affected by *Cag*A include vinculin, cortactin, and ezrin. This figure was adapted from an earlier version by Rieder *et al.* (2005).

potential ($\Delta\Psi m$) 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 *Cag*A [26] [35].

In our study, there was nostatistically significant difference regarding the *cag*A gene or *vac*A 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 *vac*A and *cag*A 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*

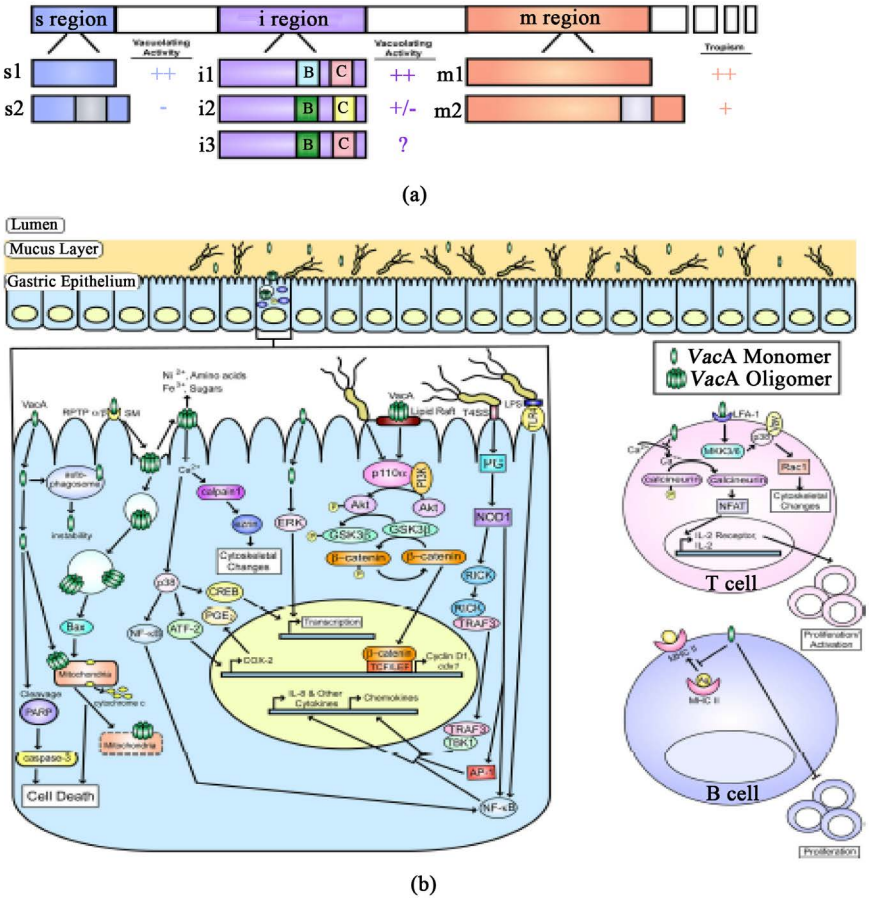

Figure 5. *Vac*A and known host cell targets [26]. (a) A schematic representation of *Vac*A with the three major regions of polymorphisms (s, i, and m) is shown. Additionally, known alleles of/corresponding to each region are shown. The i region contains two important polymorphic regions known as Cluster 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 *Vac*A 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 peptidogly can (PG) and LPS are shown. This figure was adapted from an earlier version by Rieder *et al.* (2005).

pathogenicity factors [20]. 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-

tablished [42]. *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 *cag*A positivity, *Cag*A and *Vac*A seropositivity, and/or *vacA* polymorphism contribute to disease severity, numerous studies have not found this association [26] [43].

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

In China, no association between *cag*A status and peptic ulceration orchronic gastritis was established, due likely to the high presence of *Cag*A 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 *cag*A and *vac*A 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 *cag*A and *vac*A 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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