Int.J.Curr.Microbiol.App.Sci (2023) *12*(07): 54-64

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 12 Number 7 (2023)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2023.1207.006

Characterization of *Helicobacter pylori* Virulence Genes *cagE, iceA* and *oipA* in Stool Samples from Burkina Faso

Tegwinde Rebeca Compaore[1, 2, 3]*, Yasmine Astrid Sana[3], Sylvie Zida[1], Nômawendé Ines Compaore[4], Lassina Traore[3, 2, 1], Oumarou Ouedraogo[1], Abdou Azaque Zoure[1, 2, 3], Serge Theophile Soubeiga[1, 2, 3], Dinanibe Kambire[1], Kalifou Traore[3], Tani Sagna[1, 2, 3], Florencia Wendkuuni Djigma[3, 2, 1], Henri Gautier Ouedraogo[1, 3] and Jacques Simpore[2, 3]

[1]Institut de Recherche en Sciences de la Santé (IRSS), National Centre for Scientific and Technologic Research / CNRST), Ouagadougou, Burkina Faso.
[2]Centre de Recherche Biomoléculaire Pietro Annigoni (CERBA), Ouagadougou, Burkina Faso
[3]Laboratoire de Biologie et de Génétique Moléculaire(LABIOGENE); Université Joseph KI-ZERBO, Ouagadougou, Burkina Faso
[4]Clinique El Fateh-Suka, Service de médecine et de spécialités médicales, Ouagadougou, Burkina Faso
[5]Département de Médecine et de Pharmacopée Traditionnelle, Institut de Recherche en Sciences de la Santé (IRSS), Ouagadougou, Burkina Faso

**Corresponding author*

Keywords

Helicobacter pylori, PCR, cagE, iceA, oipA, stool sample, Burkina Faso

Article Info

Received:
03 June 2023
Accepted:
05 July 2023
Available Online:
10 July 2023

A B S T R A C T

Helicobacter pylori infection leads to gastritis that can evolve into severe forms of ulceration and malignant transformation. Its diagnosis is most often invasive. Our study aim was to characterize by a non-invasive method, *Helicobacter pylori* virulence genes *iceA, oipA, and cagE* in stool samples from Burkina Faso. This cross-sectional study took place from September 2020 to February 2021 and involved 250 patients. Each patient provided a stool sample, which was used to perform an *Helicobacter pylori* antigenic detection test and molecular analysis by polymerase chain reaction (PCR) to detect its virulence genes *cagE, iceA,* and *oipA*. The results showed a *Helicobacter pylori* infection rate of 89.6%. The virulence genes *cagE, iceA1, iceA2,* and *oipA* frequencies were 75.4%, 3.12%, 3.12%, and1.78% respectively. Our bacterial population had only one *iceA* genotype. The *cagE* gene was significantly linked with *H. pylori* infection *(p-value=0.001)*. We also found a significant association between the *iceA2* gene and a risk factor, with a *(p-Value=* 0.01). Conclusion: Our study is one of the first characterizing *cagE, iceA1, iceA2,* and *oipA* virulence genes of *Helicobacter pylori* in Burkina Faso. It suggests that virulence genes can be tested in stool as an on-invasive method.

Introduction

Helicobacter pylori (*H. pylori*) is a bacterium prevalent in all countries and continents. On a global scale, its prevalence is greater than 50% (Hooi *et al.,* 2017). This bacterium is a public health problem especially, in developing countries. It is thought that it is transmitted during childhood and lasts most of the time throughout life. *H. pylori* infection leads to gastritis that can evolve into severe forms of ulceration and malignant transformation. Its main reservoir is the human stomach. The prevalence of infection varies by geographic area, age, ethnicity, and socioeconomic status (Hooi *et al.,* 2017). In fact, *Helicobacter pylori* prevalence varies in Burkina Faso between 80 and 92% according to the diagnosis method and the study population (Cataldo *et al.,* 2004; Ilboudo, 1997; Sermé, 2016; Werme *et al.,* 2015). Survival and persistence of the bacterium are related to the expression of several colonization and virulence factors (Wroblewski *et al.,* 2010). A comparison of the genome of the different sequenced strains has revealed a high genetic diversity in *H. pylori* (Sterbenc *et al.,* 2019). Although *H. pylori* infection almost inevitably leads to chronic active gastritis. Approximately 10-15% of infected individuals develop severe gastroduodenal disease. Diseases such as peptic ulcer, gastric carcinoma (GC), and mucosa-associated tissue lymphoma (MALT) are caused by *H. pylori* (Sterbenc *et al.,* 2019). The exact molecular mechanisms by which *H. pylori* infection induces a severe clinical outcome have not yet been clearly elucidated. They put into play several elements, including host genetic and environmental factors, as well as specific bacterial virulence genes, each with its own role and mechanism of action. The gene associated with cytotoxin E *(CagE*) would be associated with duodenal ulcer. *CagE* is required for the induction of interleukin (IL)-8 from gastric epithelial cells (Ozbey *et al.,* 2013). *CagE* is a component of the type IV secretion system. It is essential for translocation and phosphorylation of *cag A*. Another important determinant of *H. pylori* virulence is the Induced Contact Epithelium (*iceA*) gene. The *iceA* gene has two allelic types, *iceA1* and *iceA2*. Significantly expressed, *iceA1* is associated with peptic ulceration (Peek *et al.,* 1998). Both *iceA* alleles are bordered by, *cysE* and *hpyIM* which are conserved genes, but they differ significantly in their genetic and sequence organization (Koehler *et al.,* 2003). Expression of the *iceA* genes by the bacterium is induced upon contact of *H. pylori* with gastric epithelial cells and the *iceA1* variant is upregulated. The exact function of *iceA* peptides is still unknown, although sequence homology suggests a role in endonuclease activity, it appears to be a bacterial restriction enzyme (Peek *et al.,* 1998). Previous studies have shown that the presence of *iceA1* is associated with increased mucosal interleukin 8 concentrations, enhanced acute neutrophilic infiltration and a higher risk of developing peptic ulcer disease (Arents *et al.,* 2001; Koehler *et al.,* 2003; Shiota *et al.,* 2013). Recently, a new putative virulence factor has been identified, the outer inflammatory protein (*oipA*) gene, which encodes the inflammatory membrane protein. It is located approximately 100 kb from the pathogenicity island (PAI) cag on the *H. pylori* chromosome (Ben Mansour *et al.,* 2010). *OipA* would contribute to IL-8 secretion by epithelial cells, induction of the matrix metalloprotease 1 (MMP-1) is strongly associated with gastric cancer (Yamaoka *et al.,* 2006). The protein action is through inducing an inflammation and causing an actin dynamic that usually worth with cag PAI pathways. *OipA* would therefore interact synergistically with other *H. pylori* virulence factors to result in severe gastric injury (Yamaoka, 2012). There is a polymorphism of *oipA* and active production of *oipA* proteins. They can be "on" or "off" depending on the number of repeats of the CT nucleotide in the *oipA* gene signal sequence (HP0638). The synthesis of *oipA* is regulated by a gene phasing mechanism due to the existence of a large polymorphism at the 5' end of the gene. The presence of 6 or 9 CT dinucleotides in the 5' region of the gene allows functional expression of the gene and production of *oipA* protein, whereas the presence of 5 or 7 CTs leads to the absence of protein synthesis (Sallas *et al.,* 2019). The functional *oipA* stimulates *H. pylori* capacity of binding to the

gastric epithelium, which can be followed by host cell apoptosis, toxicity and inflammation initiation through interleukin-8 (IL-8) production, thus causing severe gastric pathology (Sterbenc *et al.*, 2019). In Sub-Saharan Africa and especially in Burkina Faso, there is a need to investigate *Helicobacter pylori* virulence factors and their implication in infection and dyspepsia. The aim of our study was to characterize the virulence genes (*cagE*, *iceA*, and *oipA*) of *H. Pylori* in stool samples.

Materials and Methods

Patient recruitment and data collection

This was a cross-sectional study that ran from September 2020 to February 2021. A total of 250 stool samples were collected. All the patients received with stool samples at the bacteriology department of the "Hôpital Saint Camille" of Ouagadougou (HOSCO), the Pietro Annigonni Biomolecular Research Center (CERBA), who wished to perform an antigenic test for *H. pylori* during the collection period and who had given their consent were considered for the study. All patients were interviewed to collect sociodemographic data (age, gender, residence, occupation) and clinical data (history, risk factors, and symptoms).

Symptoms considered were gastric and abdominal pain, bloating, gastroesophageal reflux. Family size, number of hand washings per day, water source, consumption of fresh produce, and alcohol consumption were considered as risk factors for infection.

Laboratory methods

Only stool samples from patients who were positive for *H. pylori* rRNA16s by molecular test were retained for further study for the detection of virulence genes. Samples were frozen at -20°C in cryotubes for DNA extraction. *H. pylori* DNA was extracted using the QIAamp® Fast DNA Stool Mini (Qiagen) according to the manufacturer's instructions with 220 mg of samples.

Rapid diagnostic test for H. pylori antigen

The rapid test used for antigenic detection of *H. pylori* was "The OneStep *H. Pylori* Ag Stool Rapid Test" (Innovation Biotech, Beijing). The test according to the manufacturer has 96%of specificity and over 98 % of accuracy.

Detection of virulence genes by PCR

Conventional multiplex PCR was used in our study for the amplification of *cagE* (Erzin *et al.*, 2006), *iceA1*, *iceA2* (Ben Mansour *et al.*, 2010) and *oipA* (Amjad *et al.*, 2010) genes with specific primers (see Table 1). PCR was done from a reaction volume of 25 µL, using the gene Amp®9700 thermal cycler machine (Applied Biosystems). In a previous study, a PCR was performed with the r*RNA16S* primer searched at 110 bp fragment to ensure the presence of *H. pylori* in our DNA samples (Paredes-Osses *et al.*, 2017).

PCR multiplex *cagE, iceA1, OipA, iceA2*

For the *cagE, iceA1* multiplex we used for the preparation of the reaction mixture 12 µL of the diluted Master mix (1.5X), 0.5 µL of each primer (sense and antisense 0.1X), 6 µL of sterile water and 5 µL of the DNA extract of each sample. The PCR program included an initial denaturation cycle at 94°C for 5 minutes, followed by 35 cycles including denaturation at 94°C for 60 seconds, hybridization at 55°C for 60 seconds and elongation at 72°C for 1 minute. The final cycle included a 5min extension step at 72°C.

The *oipA, iceA2* multiplex reaction contained of 12 µL of diluted Master mix (1.5X), 0.5 µL of each primer (sense and antisense 0,05X), 6 µL of sterile water, and 5 µL of DNA extract from each sample. The PCR program included an initial denaturation cycle at 95°C for 5 minutes, followed by 35 cycles including denaturation at 95°C for 60 seconds, hybridization at 55°C for 60 seconds and elongation at 72°C for 1 minute. The final cycle included a 5 min extension step at 72°C to ensure complete

extension of the PCR products, which were migrated in a 2% agarose gel by electrophoresis.

Statistical analysis

Clinical data were first entered into Excel version 2016. Then the analysis was done using the standard software Statistical Package for Social Sciences version 25(SPSS, Inc., Chicago, IL). The chi-square test was calculated using two by two table to evaluate the link between *Helicobacter pylori* infection, virulence genes and with epidemiological factors. We set the statistical significance level at ($p < 0.05$).

Ethical considerations

All patients and guardians of included patients had consented to participate in the study. This study obtained the approval of the Ethics Committee for Health Research of Burkina Faso (Deliberation n° 2020-12-274). All patients were subjected to a questionnaire concerning socio-demographic data (age, sex, occupation, place of residence) and medical history. Their answers were kept anonymous.

Results and Discussion

Sociodemographic and lifestyle characteristics of the population

The youngest patient was 4 years old and the oldest 80 years old, and the mean age was 38.6 ± 15 years. The most represented age group was [20-40] years with a frequency of 55.6% followed by [40-60] years with a frequency of 26.8%.

Most patients were female with a frequency of 57.6%. The sex ratio was 0.7 in favor of women. The most represented professional class was the informal sector with a frequency of 61.2%. Almost all the patients lived in urban areas with a frequency of 96.8%. The distribution of patients according to lifestyle showed that most patients washed their hands less than or equal to 3 times a day with a percentage of 68%. Also, 82.8% of patients did not report alcohol consumption and 98% drank tap water, often alternating with borehole water.

The patients' diet was quite diverse. Those who took their meals alone represented 76.4%. The number of people living in families with less than or equal to 5 people in the same habitat had a percentage of 62.4%.

Detection of *Helicobacter pylori* virulence genes

Prevalence of *H. Pylori* infection

Helicobacter pylori prevalence in our study population was 89.9% (224/250). The *cagE* virulence gene was identified in 169 of the positive patients (75.4%) (Table 3). The presence of the *cagE* gene was linked to *H. pylori* infection (p-value =0.0001) (Table 3).

The *iceA1* and *iceA2* were detected among seven (7) patients each with frequencies of 3.12% and the *oipA* gene was detected among 1.78% of patients.

Association between virulence genes, risk factors, and clinical signs

We found a significant association between the *iceA2* gene and the water source, with a *p-Value* of *0.01*(Table 4). We also found a significant association between the virulence gene *iceA2*, and the existence of symptoms related to the infection with a *p-Value* of 0.04 (Table 5).

Relationship between virulence genes and treatment in symptomatic patients

A significant association between virulence genes and treatment was observed. We noted that there was a relationship between the presence of the *cagE* gene and *Helicobacter pylori* treatment with a p-*value* of *0.04* (Table 6).

There was no significant association between the presence of multiple virulence genes and *H. pylori*

infection (supplementary Table 1). Two patients had three virulence genes (*cagE, iceA1, oipA*) or (*cagE, iceA2, oipA*). None of the patients had all four virulence genes. The two allelic forms of the *iceA* gene were not found in the same patient in our study.

This study is one of the first to screen for *Helicobacter pylori* for virulence genes *cagE, iceA,* and *oipA* in stools in West Africa. The most represented age group was the age group from 20 to 30 with 49.2% positive cases; the least defined age group was the under 20 years with 7.6%. However, the under-20 age group had a higher infection rate of 94.7% (18 positive cases out of 19 total).

Our results are comparable to those of Ilboudo *et al.,* (1997) and Werme *et al.,* (2015) who also observed a low representation of infection in patients under 20 years of age. This low representation in patients younger than 20 years could be explained by the fact that patients of this age do not show enough symptoms of infection (Wroblewski *et al.,* 2010). Many of the patients were symptomatic. The present study hypothesis is that the symptoms of *H. pylori* infection occur in young adults.

At this age, they are persistent enough to require a consultation, hence the investigation for *Helicobacter pylori*. Thus, contamination would occur in young children to reach a high prevalence in adult life; it has been shown that *H. pylori* could survive for decades in the human stomach in an asymptomatic manner (without clinical manifestations). Indeed, acquisition of the bacterium in childhood is accompanied in most cases by chronic gastritis, which can evolve without further complications and remain asymptomatic (Megraud *et al.,* 2015).

Occupation and standard of living were not significantly associated with *H. pylori*. Our results are consistent with those of Serme *et al.,* (2016). In fact, it is thought that "in Africa, any individual taken in adulthood and whatever his socio-economic level, has experienced a childhood in an environment conducive to contamination". Indeed, several studies have shown that contamination occurs early in childhood and before the age of 10, more than 50% of children in developing countries are already contaminated (Ilboudo, 1997). This could explain the absence of significant differences regardless of the socio-demographic factor considered (age, sex, profession, standard of living).

However, the frequency of infection was higher at 90.58% in patients who did not wash their hands more than three times a day. Also, patients who lived with five or more people in the same household had an infection rate of 90.4%. These results support those in the literature that lack of hygiene, crowding in families would be risk factors for *H. Pylori* infection or re-infection (Ben Mansour *et al.,* 2010; Hooi *et al.,* 2017; Ofori *et al.,* 2019).

The *cagE* gene was detected among 75.44% of the patients positive for H. pylori. These results are higher than those obtained in Turkey by Ozbey *et al.,* (2013) in gastric biopsy samples at 33.3%.

This difference could be explained by the geographical arrangement of the countries or by the type of sampling. Our results are consistent with those of Yamazaki *et al.,* (2005) in Japan who found that the *cagE* gene was well conserved among the studied strains.

The present study results are also comparable to those Erzin *et al.,* (2006) who found a prevalence of 59.3% in only 91 cases. They found a significant association of the gene with cases of duodenal ulcer and gastric cancer. We found a significant association between *H. pylori* infection and the presence of the *cagE* gene, and a significant association between no treatment and the presence of the *cagE* gene.

We can deduce that the absence of treatment would contribute to the persistence of virulence genes during infection. The presence of the *cagE* gene has been associated with a poor prognosis of the infection, particularly in some countries (Tan *et al.,* 2005).

Table.1 Primers sequence and size in base pair

Genes	Primer sequence (5'-3')	Size(pb)	Reference
rRNA16s	F: CTCGAGAGACTAAGCCCTCC R: ATTACTGACGCTGAT GTGC	110	(Paredes-Osses *et al*., 2017)
OipA	F: GTTTTTGATGCATGGGATTT R: GTTTTTGATGCATGGGATTT	401	(Amjad *et al*., 2010)
CagE	F: GCGATTGTTATTGTGCTTGTAG R: GAAGTGGTTAAAAAATCAATGCCCC	329	(Erzin *et al*., 2006)
IceA1	F: GTGTTTTTAACCAAAGTATC R: CTATAGCCASTYTCTTTGCA	247	(Ben Mansour *et al*., 2010)
IceA2	F: GTTGGGTATATCACAATTTAT R: TTRCCCTATTTTCTAGTAGGT	229 or 334	(Ben Mansour *et al*., 2010)

Table.2 Distribution of the study by socio-demographic characteristics

Characters	Category	Effectives	Percentage (%)
Age (years)	≤20	19	7.6
	]20-40]	139	55.6
	]40-60]	67	26.8
	>60	25	10.0
Sex	Male	106	42.4
	Female	144	57.6
Profession	Pupils/Students	63	25.2
	Officials	34	13.6
	Informal sector	153	61.2
Residence	Urban	242	96.8
	Rural	8	3.2

Table.3 Relation between virulence genes *cagE*, *iceA1*, *iceA2*, and *oipA* with *Helicobacter pylori* infection

Virulence genes	Category	*H. pylori*			
		Absent n (%)	**Present n (%)**	**Total n (%)**	**p-value**
CagE	Absent	26 (10.40)	55 (22)	81 (32.40)	**0.0001**
	Present	0 (0.00)	169 (67.60)	169 (67.60)	
IceA1	Absent	26 (10.40)	217 (86.8)	243 (97.2)	0.36
	Present	0 (0.00)	7 (2.80)	7 (2.80)	
IceA2	Absent	26 (10.40)	217 (86.8)	243 (97.2)	0.36
	Present	0 (0.00)	7 (2.80)	7 (2.80)	
OipA	Absent	26 (10.40)	220 (88)	246 (98.4)	0.49
	Present	0 (0.00)	4 (1.6)	4 (1.6)	

Table.4 Relationship between the *iceA2* gene and risk factors

Risk factors	Category	*IceA2*			
		Absent n (%)	Presence n (%)	Total n (%)	p-value
Age (years)	≤20	19 (7.6)	0 (0)	19 (7.6)	0.58
	]20-40]	135 (54)	4 (1.78)	139 (55.60)	
	]40-60]	64 (25.60)	3 (1.2)	67 (26.80)	
	>60	25 (10)	0 (0)	25 (10)	
Number Handwashing Per day	>3	79 (31.60)	1 (0.4)	80 (32)	0.30
	≤3	164 (65.60)	6 (2.4)	170 (68)	
Water source	Tap water	239 (95.60)	6 (2.4)	245 (98)	**0.01**
	Other	4 (1.6)	1 (0.4)	5 (2)	
Consumption/ Fresh products	No	193 (77.2)	5 (2)	198 (79.2)	0.60
	Yes	50 (20)	2 (0.80)	52 (20.80)	
Alcohol consumption	No	202 (80.80)	5 (2)	207 (82.20)	0.41
	Yes	41 (16.4)	2 (0.80)	43 (17.2)	

Table.5 Correlation of virulence genes *cagE, iceA1, iceA2, oipA* and clinical signs

Virulence genes	Category	Clinical signs			
		Asymptomatic n (%)	Symptomatic n (%)	Total n (%)	p-value
CagE	Absent	6 (2.67)	75 (33.48)	81 (36.10)	0.8
	Present	14 (5.60)	155 (69.19)	230 (74.79)	
IceA1	Absent	20 (8)	223 (89.20)	243 (97.2)	0.4
	Present	0 (0)	7 (3.12)	7 (2.8)	
IceA2	Absent	18 (7.2)	225 (90)	243 (97.2)	**0.042**
	Present	2 (0.80)	5 (1.78)	7 (2.80)	
OipA	Absent	20 (8)	226 (90.40)	246 (98.2)	0.5
	Present	0 (0)	4 (1.60)	4 (1.60)	

Table.6 Relationship of virulence genes *cagE, iceA1, iceA2, oipA* among patients with symptoms and *H. pylori* treatment

	Category	*H. pylori* treatment			
		No treatment n (%)	Treatment n (%)	Total n (%)	p-value
CagE	Absent	45 (22.05)	4 (1.96)	49 (24.01)	**0.046**
	Present	123 (60.29)	32 (15.68)	155 (75.98	
IceA1	Absent	162 (79.41)	35 (17.15)	197 (96.56)	0.81
	Present	6 (2.94)	1 (0.49)	7 (3.43)	
IceA2	Absent	163 (79.90)	36 (17.64)	199 (97.54)	0.29
	Present	5 (2.45)	0 (0)	5 (2.45)	
OipA	Absent	164 (80.39)	36 (17.64)	200 (98.03)	0.35
	Present	4 (1.96)	0 (0)	4 (1.96)	

Table.7 Simultaneous Present of virulence genes *cagE, ice A,* and *oipA* in relation to *Helicobacter pylori* infection

Virulence genes	Category	*H. pylori*			
		Absent n (%)	Present n (%)	Total n (%)	p-value
CagE+IceA1	Absent	26(10.40)	218(87.2)	244(97.60)	0.39
	Present	0(0)	6(2.4)	6(2.4)	
CagE+IceA2	Absent	26(10.40)	219(87.60)	245(98)	0.44
	Present	0(0)	5(2)	5(2)	
CagE + OipA	Absent	26(10.40)	222(88.8)	248(99.2)	0.62
	Present	0(0)	2(0.80)	2(0.80)	
IceA1+IceA2	Absent	26(10.40)	224(89.6)	250(100)	-
	Present	0(0)	0(0)	0(0)	
IceA1 + OipA	Absent	26(10.40)	223(89.8)	249	0.73
	Present	0(0)	1(0.4)	1(0.4)	
IceA2 + OipA	Absent	26(10.4)	223(89.2)	249(99.6)	0.73
	Present	0(0)	1(0.4)	1(0.4)	
CagE+ IceA +OipA	Absence	26(10.4)	223(89.2)	249(99.6)	0.73
	Present	0(0)	1(0.4)	1(0.4)	

In this study the *iceA* gene was detected at 3.12% and the frequency of both *iceA1* and *iceA2* alleles was identical. Our results are lower than those of Smith *et al.*, who found that a frequency of 86.4% of *iceA*1 among patients from Nigeria with non-ulcer dyspepsia (Ofori *et al.*, 2019). Erzin *et al.*, (2006) obtained 74% for *iceA1* and 25.3% for *iceA2* and those of Ben Mansour *et al.*, (2010) who obtained 60.2% for *iceA1* and 16% for *iceA2*. No patient had both allelic forms of *iceA* in our study population. These results are consistent with those of Erzin *et al.*, (2006) in Turkey and different with those of Amjad *et al.*, (2010) in Malaysia who found an association between the two allelic forms (Amjad *et al.*, 2010; Erzin *et al.*, 2006).

Thus, the present study bacterial population seem to possess a single *iceA* allelic form. This discrepancy in frequency could be explained by the fact the difference in study populations and virulence genes detection method also, Additionally, *iceA* genes were found to not be well conserved in the stool (Monteiro *et al.*, 2001). We found a significant association between the presence of the *iceA2* gene and the type of water source and with clinical signs. The water source might be a source of infection for this virulence gene. Furthermore, the presence of symptoms could be indicative of the presence of the *iceA2* gene in our study population.

However, further studies are needed to confirm these hypotheses. Peek *et al.*, detected higher acute inflammation in the gastric mucosa of patients colonized with *iceA1* positive strains (Erzin *et al.*, 2006), a finding that may explain the association between this genotype and ulcer. However, the *iceA1* genotype does not always relate to ulcer (Shiota *et al.*, 2012).

The *oipA* gene was the lowest detected gene in our study with a prevalence of 1.78%. This is different from the result of El-Sayed *et al.*, (2020) in Egypt who found a frequency of 32.5% and that of Ben Mansour *et al.*, in Tunisia which was 90.8% (Ben Mansour *et al.*, 2010; El-Sayed Marwa Shabban, 2020). Our results are also lower than those of Ozbey *et al.*, (2013) who found 75% in case of gastritis and 85.7%, in case of peptic ulcer (Ozbey *et al.*, 2013). These results can be explained by the difference sample types, the bacterial strain, study

population and the and by the fact that *oipA* gene may be less common in our bacterial population.

Yamakoa *et al.*, (2012) showed that the presence of the *oipA* gene in a functional form was significantly associated with the presence of a high bacterial load in the gastric mucosa of infected patients with an increase in the infiltration of the mucosa by neutrophils (Yamaoka, 2012).

The presence of the functional *oipA* gene increases the risk of duodenal ulcer and gastric cancer (Liu *et al.*, 2013). Several studies in the literature have shown that the *oipA* gene is associated with peptic ulcer disease. Two of our patients (0.89%) had combined genotypes of three virulence genes. The genes involved were the *cagE* gene, the *iceA1* gene for the first patient and the *iceA2* gene for the second patient, and finally the *oipA* gene.

These patients would be more likely to develop complications such as adenocarcinoma or gastric lymphoma (Koehler *et al.*, 2003). Our study has some limitations such as the sample size and the fact that we lacked information on the different stages of *Helicobacter pylori* infection.

This study allowed us to characterize the virulence genes and alleles *iceA1*, *iceA2*, *oipA*, *cagE* of *H. pylori* in stool samples. It also reports for one of the first time the presence of Helicobacter virulence genes *cagE* and *oipA* on samples from West Africa. Molecular methods can be used to detect *Helicobacter pylori* virulence genes in stool samples.

Data Availability Statement

The data supporting this study's findings are available upon request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Conflict of Interests

The authors declare that there is no conflict of interest.

Funding

This study was supported by "The World of Science Academy" (TWAS, grant: 20-080 RG/BIO/AF/AC), the « Institut de Recherche en Sciences de la Santé » / « Centre National de Recherche Scientifique et Technologique » (IRSS/SCNRST) and the « Laboratoire de Biologie et de Génétique Moléculaire » (LABIOGENE), Université Joseph KI-ZERBO.

Acknowledgments

We thank the technical staff of the "Centre de Recherche Biomoléculaire Pietro Annigoni" for the excellent technical assistance with the sample collection.

Authors' contributions

TRC and YAS conceived and designed the experiments and wrote the manuscript; TRC, YAS, SZ, NIC, LT, STS, DK, KT, and TS performed the experiments; WFG and HGO supervised the research and finalized the manuscript. JS contributed to the study design, experimental assays, writing, and critical reviewing of the content and approved the final version of the manuscript. All authors have read and agreed to the published version of the manuscript.

References

Amjad, N., Osman, H. A., Razak, N. A., Kassian, J., Din, J., and bin Abdullah, N. (2010). Clinical significance of *Helicobacter pylori cagA* and *iceA* genotype status. World J Gastroenterol *16*, 4443-4447. https://doi.org/10.3748/wjg.v16.i35.4443

Arents, N. L., van Zwet, A. A., Thijs, J. C., Kooistra-Smid, A. M., van Slochteren, K. R., Degener, J. E., Kleibeuker, J. H., and van Doorn, L. J. (2001). The importance of *vacA*, *cagA*, and *iceA* genotypes of *Helicobacter pylori* infection in peptic ulcer disease and gastroesophageal reflux disease. Am J Gastroenterol *96*, 2603-2608. https://doi.org/10.1111/j.1572-

0241.2001.04104.x

Ben Mansour, K., Fendri, C., Zribi, M., Masmoudi, A., Labbene, M., Fillali, A., Ben Mami, N., Najjar, T., Meherzi, A., Sfar, T., *et al.*, (2010). Prevalence of *Helicobacter pylori vacA*, *cagA*, *iceA* and *oipA* genotypes in Tunisian patients. Ann Clin Microbiol Antimicrob *9*: 10. https://doi.org/10.1186/1476-0711-9-10

Cataldo, F., Simpore, J., Greco, P., Ilboudo, D., and Musumeci, S. (2004). *Helicobacter pylori* infection in Burkina Faso: an enigma within an enigma. Dig Liver Dis *36*, 589-593. https://doi.org/10.1016/j.dld.2004.05.005

El-Sayed Marwa Shabban, M. N., Eltabbakh Mohamed, Abdelhamid Dalia Hosni, Mostafa Ismail Mostafa Shimaa, Salah Manar Mohamed, Ahmed Faheem Faheem Heba & Rania Ahmed Hassan (2020). Detection of *Helicobacter pylori oipA* and *dupA* genes among dyspeptic patients with chronic gastritis. Alexandria Journal of Medicine *56*, 6. https://doi.org/10.1080/20905068.2020.1780675

Erzin, Y., Koksal, V., Altun, S., Dobrucali, A., Aslan, M., Erdamar, S., Dirican, A., and Kocazeybek, B. (2006). Prevalence of *Helicobacter pylori vacA*, *cagA*, *cagE*, *iceA*, *babA2* genotypes and correlation with clinical outcome in Turkish patients with dyspepsia. Helicobacter *11*, 574-580. https://doi.org/10.1111/j.1523-5378.2006.00461.x

Hooi, J. K. Y., Lai, W. Y., Ng, W. K., Suen, M. M. Y., Underwood, F. E., Tanyingoh, D., Malfertheiner, P., Graham, D. Y., Wong, V. W. S., Wu, J. C. Y., *et al.*, (2017). Global Prevalence of *Helicobacter pylori* Infection: Systematic Review and Meta-Analysis. Gastroenterology *153*, 420-429. https://doi.org/10.1053/j.gastro.2017.04.022

Ilboudo D, S. L., Sanou J, Bougma A, Diomande I (1997). Aspects epidemiologiques et Cliniques de l'Infection a *Helicobacter pylori* en zone tropicale : A propos de 150 patients a L'hopital national de Ouagadougou (Burkina Faso). Médecine d'Afrique Noire *44*, 5.

Koehler, C. I., Mues, M. B., Dienes, H. P., Kriegsmann, J., Schirmacher, P., and Odenthal, M. (2003). *Helicobacter pylori* genotyping in gastric adenocarcinoma and MALT lymphoma by multiplex PCR analyses of paraffin wax embedded tissues. Mol Pathol *56*, 36-42. https://doi.org/10.1136/mp.56.1.36

Liu, J., He, C., Chen, M., Wang, Z., Xing, C., and Yuan, Y. (2013). Association of presence/absence and on/off patterns of *Helicobacter pylori oipA* gene with peptic ulcer disease and gastric cancer risks: a meta-analysis. BMC Infect Dis *13*, 555. https://doi.org/10.1186/1471-2334-13-555

Megraud, F., Bessede, E., and Varon, C. (2015). *Helicobacter pylori* infection and gastric carcinoma. Clin Microbiol Infect *21*, 984-990. https://doi.org/10.1016/j.cmi.2015.06.004

Monteiro, L., Gras, N., Vidal, R., Cabrita, J., and Megraud, F. (2001). Detection of *Helicobacter pylori* DNA in human feces by PCR: DNA stability and removal of inhibitors. J Microbiol Methods *45*, 89-94. https://doi.org/10.1016/s0167-7012(01)00225-1

Ofori, E. G., Adinortey, C. A., Bockarie, A. S., Kyei, F., Tagoe, E. A., and Adinortey, M. B. (2019). *Helicobacter pylori* Infection, Virulence Genes' Distribution and Accompanying Clinical Outcomes: The West Africa Situation. Biomed Res Int *2019*, 7312908. https://doi.org/10.1155/2019/7312908

Ozbey, G., Demirel, U., Aygun, C., and Ertas, H. B. (2013). Investigation of the association between clinical outcome and the cag pathogenicity-island and other virulence genes of *Helicobacter pylori* isolates from patients with dyspepsia in Eastern Turkey. Braz J Microbiol *44*, 1267-1274. https://doi.org/10.1590/s1517-83822013000400034

Paredes-Osses, E., Saez, K., Sanhueza, E., Hebel, S., Gonzalez, C., Briceno, C., and Garcia Cancino, A. (2017). Association between *cagA*, *vacAi*, and *dupA* genes of *Helicobacter pylori* and

gastroduodenal pathologies in Chilean patients. Folia Microbiol (Praha) *62*, 437-444. https://doi.org/10.1007/s12223-017-0514-y

Peek, R. M., Jr., Thompson, S. A., Donahue, J. P., Tham, K. T., Atherton, J. C., Blaser, M. J., and Miller, G. G. (1998). Adherence to gastric epithelial cells induces expression of a *Helicobacter pylori* gene, *iceA*, that is associated with clinical outcome. Proc Assoc Am Physicians *110*, 531-544.

Sallas, M. L., Dos Santos, M. P., Orcini, W. A., David, E. B., Peruquetti, R. L., Payao, S. L. M., and Rasmussen, L. T. (2019). Status (on/off) of *oipA* gene: their associations with gastritis and gastric cancer and geographic origins. Arch Microbiol *201*, 93-97. https://doi.org/10.1007/s00203-018-1580-5

Sermé A K, C. N., Compaoré R, Djigma F, Somda K S, Diarra B, Coulibaly A, Zohoncon T, Obiri-Yeboah D, Sombie A R, Bougouma A and Simporé J (2016). *Helicobacter pylori* and upper digestive diseases - Diagnosis through Real Time PCR. Nigerian Journal of Gastroenterology and Hepatology *8*, 71 -80.

Shiota, S., Suzuki, R., and Yamaoka, Y. (2013). The significance of virulence factors in *Helicobacter pylori*. J Dig Dis *14*, 341-349. https://doi.org/10.1111/1751-2980.12054

Shiota, S., Watada, M., Matsunari, O., Iwatani, S., Suzuki, R., and Yamaoka, Y. (2012). *Helicobacter pylori iceA*, clinical outcomes, and correlation with *cagA*: a meta-analysis. PLoS One *7*, e30354. https://doi.org/10.1371/journal.pone.0030354

Sterbenc, A., Jarc, E., Poljak, M., and Homan, M. (2019). *Helicobacter pylori* virulence genes. World J Gastroenterol *25*, 4870-4884. https://doi.org/10.3748/wjg.v25.i33.4870

Tan, H. J., Rizal, A. M., Rosmadi, M. Y., and Goh, K. L. (2005). Distribution of *Helicobacter pylori cagA*, *cagE* and *vacA* in different ethnic groups in Kuala Lumpur, Malaysia. J Gastroenterol Hepatol *20*, 589-594. https://doi.org/10.1111/j.1440-1746.2005.03783.x

Werme, K., Bisseye, C., Ouedraogo, I., Yonli, A. T., Ouermi, D., Djigma, F., Moret, R., Gnoula, C., Nikiema, J. B., and Simpore, J. (2015). [Molecular diagnostics of *Helicobacter pylori* by PCR in patients in gastroenterology consultation at Saint Camille Medical Centre in Ouagadougou]. Pan Afr Med J *21*, 123. https://doi.org/10.11604/pamj.2015.21.123.6001

Wroblewski, L. E., Peek, R. M., Jr., and Wilson, K. T. (2010). *Helicobacter pylori* and gastric cancer: factors that modulate disease risk. Clin Microbiol Rev *23*, 713-739. https://doi.org/10.1128/CMR.00011-10

Yamaoka, Y. (2012). Pathogenesis of *Helicobacter pylori*-Related Gastroduodenal Diseases from Molecular Epidemiological Studies. Gastroenterol Res Pract *2012*, 371503. https://doi.org/10.1155/2012/371503

Yamaoka, Y., Ojo, O., Fujimoto, S., Odenbreit, S., Haas, R., Gutierrez, O., El-Zimaity, H. M., Reddy, R., Arnqvist, A., and Graham, D. Y. (2006). *Helicobacter pylori* outer membrane proteins and gastroduodenal disease. Gut *55*, 775-781. https://doi.org/10.1136/gut.2005.083014

How to cite this article:

Tegwinde Rebeca Compaore, Yasmine Astrid Sana, Sylvie Zida, Nômawendé, Ines Compaore, Lassina Traore, Oumarou Ouedraogo, Abdou Azaque Zoure, Serge Theophile Soubeiga, Dinanibe Kambire, Kalifou Traore, Tani Sagna, Florencia Wendkuuni Djigma, Henri Gautier Ouedraogo and Jacques Simpore. 2023. Characterization of *Helicobacter pylori* Virulence Genes *cagE, iceA* and *oipA* in Stool Samples from Burkina Faso. *Int.J.Curr.Microbiol.App.Sci.* 12(07): 54-64.

doi: https://doi.org/10.20546/ijcmas.2023.1207.006