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Helicobacter pylori Virulence Genes *cagA*, *babA2*, and *vacA* Detection in Dyspeptic Patients from Burkina Faso

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Abstract

The diverse clinical presentation of *Helicobacter pylori* (*H. pylori*) infection results from the interaction between bacterial virulence, host genetics, socio-demographic and environmental factors. This study aimed to characterize *Helicobacter pylori* virulence genes and the associated behavioral factors among dyspeptic patients in Burkina Faso. Two hundred and fifty (250) stool samples were collected from patients with dyspepsia seen at health centers in Ouagadougou, Burkina Faso. Bacterial deoxyribonucleic acid (DNA) was extracted using a commercial kit. Virulence genes were detected using conventional multiplex Polymerase Chain Reaction with specific primers. The overall prevalence of *Helicobacter pylori* of the 250 participants was 91.20%. *CagA* virulence gene was present among 20.19% of individuals, while *babA2* and *vacA* were detected respectively among 9.65% and 67.54% of the population positive for *Helicobacter pylori*. Among *vacA* subtypes, vacAs1 was the most frequent, with 39.04%, followed by *vacAi1* (19.74%), *vacAi2* (17.54%), and *vacAs2* with 10.96%. Regarding *vacAm1* and *vacAm2*, they were less frequent at 6.14% each. "Handwashing three times or less per day" significantly increased the risk of having *vacAi2* allele and *H. pylori* rRNA16s, with p-values of 0.013 and 0.020, respectively. The consumption of non-tap water increases the risk of carrying the *cagA* virulence gene. Additionally, *H. pylori*-positive

patients living with more than four (4) people in their household had about two times the risk of having the *vacAs1* allele. The present study shows the detection of *Helicobacter pylori cagA*, *vacA* subtypes, and *babA2* by stool a PCR method in Burkina Faso. The strong association between sanitary habits and virulence factors depicts the composite interaction between ecological factors, gastric mucosa, and bacteria. Therefore, the synergic action of these factors should be considered when aiming for bacterial eradication and gastric pathology cure.

Keywords

Helicobacter pylori, Stool, *cagA*, *babA2*, *vacA*

1. Introduction

Helicobacter pylori (*H. pylori*), a coiled mobile and microaerophilic, is a Gram-negative bacterium that colonizes the human host stomach, where it causes inflammation and affects gastric physiology. The overall prevalence of *H. pylori* infection is close to 50 percent, with Africa bearing about 70% of this prevalence, followed by South America at 69.4% and Western Asia at 66.6% [1]. The high rate of *Helicobacter pylori*, especially in emerging countries, is probably due to the transmission mode, such as direct contact between family members and consuming contaminated food and water [2]. In Burkina Faso, *H. pylori* rate is high and varies between 80% - 92% according to the study population and the diagnostic method [3] [4] [5]. It is known that gastritis, peptic ulcer disease, mucosa-associated lymphoid tissue lymphoma, and gastric cancer are caused by *H. pylori* [6] [7]. Gastric cancer represents the twelfth most common cancer in Africa [8]. The bacteria virulence genes have been associated with the latter diseases. Among these genes, *cagA* is the most frequent and essential of the cag pathogenicity Island (PAI) of the genes linked to *H. pylori* cytotoxin, it is also an oncoprotein because of its multiple associations with gastric cancer [9]. The Cag (PAI) synthesizes type IV secretion (T4SS), which injects oncoprotein *cagA* into the host epithelial cells. *H. pylori* strains expressing *cagA* have been associated with gastroduodenal ulcers and gastric cancer [10] [11].

The *vacuolating cytotoxin A* (*vacA*) can induce vacuole formation in eukaryotic cells. It is also found in most *H. pylori* strains. *VacA* is composed of three (3) main regions: the signal (s1 and s2), intermediate (i1 and i2), and middle region (m1 and m2). It is associated with effects like proliferation inhibition and induction of apoptosis in gastric cells. *H. pylori* vacuolating activity is linked to *vacA* genotypes (s1/m1, s1/m2, and s2/m2) [12]. The presence of *cagA* is often associated with the genotype s1/i1/m1 of *vacA* [13].

Additionally, the *blood group antigen binding adhesin* (*babA*) is encoded by the *babA2* gene. It is located on the outer membrane of *H. pylori* as a principal adhesin. It identifies as the blood group antigens Lewis b on the host gastric epi-

thelium and characterizes *H. pylori* colonization density. Its presence correlates with *cagA* and *vacA* by increasing infection complications [14] [15]. Furthermore, upon attachment to the gastric epithelia favored by *babA*, *H. pylori* expresses virulent proteins *cagA* and *vacA* to escape the host immune systems. *VacA* and *cagA* can work together, *vacA* causing autophagy which allows *cagA* to accumulate in the cells [16]. The importance of *Helicobacter pylori* in the occurrence of gastroduodenal diseases and gastric cancer, there is a need to provide information on its virulence genes in Burkina Faso in the context of a low-income country. The hypothesis of hygiene and virulence subtypes of *Helicobacter Pylori* correlation is that virulence genes as part of the bacteria would be transmitted mainly through contaminated food and water. *Helicobacter pylori* virulence genes enable the bacteria to successfully colonize the gastric mucosa and allow persistent infection, which would cause inflammation and tissue damage. This study aimed to characterize *Helicobacter pylori* virulence genes and the associated behavioral factors among dyspeptic patients in Burkina Faso.

2. Material and Methods

2.1. Study Population and Sampling

The study population comprised two hundred and fifty (250) patients suffering from dyspepsia. The laboratories of the Saint Camille Hospital and the Pietro Annigoni Biomolecular Research Center (CERBA) were the settings where the patients were consecutively recruited between January and April 2020. A medical doctor prescribed a stool exam suspecting an *H. pylori* infection. The stool sampled was conserved at −80˚C after being resuspended in DNase-free water.

2.2. DNA Extraction

Bacterial DNA was extracted from stool samples using a commercial kit (QIAamp DNA Stool Mini Kit, Qiagen, Hilden, Germany) according to the manufacturer's instructions. The DNA quantity and purity were measured using a Biodrop before running the PCR assay.

2.3. Molecular Detection of *H. pylori* and Its Virulence Genes

CagA, *babA2*, *vacA* (s1, s2, i1, i2, m1, m2) virulence genes were detected by multiplex Polymerase Chain Reaction (PCR) with sets of primers per PCR, *H. pylori* rRNA16s gene using specific primers (**Table 1**). The latter gene, rRNA16s, was used to confirm the effective presence of *H. pylori* DNA in the samples. The PCR reaction master mixes were prepared in a final volume of 25 µL containing 12 µL of 1.5x FIREPol® Master Mix, 2 µL of primers (0.5 µL of each sense and antisense primer), 6 µL of sterile H_2O and 5 µL of each DNA sample. A PCR program was used for the amplification and consisted of: 94˚C for 5 min, followed by 35 cycles at 94˚C for the 30 s, 57˚C for 45 s, 72˚C for 1 min, and finally, a final extension at 72˚C for 7 min on the GeneAmp PCR System 9700 (Applied Biosystems).

Table 1. *Helicobacter pylori* virulence genes sequences.

Genes	Primer	Sequence (5'→3')	Size (bp)	Ref
rRNA16s	F R	CTCGAGAGACTAAGCCCTCC ATTACTGACGCTGAT GTGC	110	[17]
cagA	F R	GATAACAGGCAAGCTTTGAGG CTGCAAAGATTGTTTGGGAGA	349	[18]
babA2	F R	CTGCAAAAGAATGTTTGGCAG AATCCAAAAAGAAGAAAAAGTATGAAA	812	[19]
vacAs1	F R	GTCAGCATCACACCGCAAC CTGCTTGAATGCGCCAAC	259	[18]
vacAs2	F R	GCTAACACGCCAAATGATCC3' CTGCTTGAATGCGCCAAAC3'	286	[17]
vacAi1	F R	GTTGGGATTGGGGGAATGCCG TTAATTTAACGCTGTTTGAAG	426	[17]
vacAi2	F R	GTTGGGATTGGGGGAATGCCG GATCAACGCTCTGATTTGA	432	[17]
vacAm1	F R	GGTCAAAATGCGGTCATGG CCATTGGTACCTGTAGAAAC	290	[20]
vacAm2	F R	GGAGCCCCAGGAAACATTG CATAACTAGCGCCTTGCAC	352	[20]

F: forward; **R**: reverse; **bp**: base pair.

2.4. Statistical Analysis

The collected data were analyzed with SPSS version 25 software (SPSS, Inc., Chicago, IL). Two by-to-table statistics and a chi-square test were run to determine associations between *H. pylori* virulence genes, and behavioral factors. A binomial logistic regression test was also run to appreciate the link between *H. pylori* virulence genes and some risk factors. A p-*value* < 0.05 was considered statistically significant.

2.5. Ethics

The study obtained the approval of the Ethics Committee for Health Research of Burkina Faso (Deliberation n˚ 2020-12-274). All participants or guardians of participants gave their free and informed consent. Confidentiality and anonymity of the information provided were respected.

3. Results

The overall prevalence of *H. pylori* among the 250 participants was 91.2% (228/250). The patients recruited ranged in age from 4 to 80 years, with an average age of 38.56 ± 15 years. The age range of [20]-[40] represented 55.60% of the study population. Women were also the most described in our study (57.60%). Most patients resided in urban areas (96.80%), and the majority were from the

informal sector (61.20%). *H. pylori* were present among 67.36% (97/144) of the women, while it was present among 78.30% (83/106) of the males with p = 0.057.

3.1. *CagA*, *babA2*, and *vacA* Virulence Genes Detection in Stool Positive for *Helicobacter pylori*

CagA virulence gene was present among 20.19% of individuals, while *babA2* and *vacA* were detected respectively among 9.65% and 67.54% (Table 2).

Figure 1 illustrates the different frequencies of *vacA* subtypes. Among *vacA* subtypes, *vacAs1* was the most frequent, with 39.04%, followed by *vacAi1* (19.74%), *vacAi2* (17.54%), and *vacAs2* with 10.96%. Regarding *vacAm1* and *vacAm2*, they were less frequent at 6.14% each.

Genotype-wise, *vacAm2s1* was the most frequent, with 4.82% (11/228), followed by *vacAm1s1* at 3.5% (8/228). Furthermore, *vacA* genotype *m2s1i1* was 2.2% (5/228) while *vacAm2s2* and *vacAm2s2i2* genotypes frequencies were 1.3% (3/228) each, followed by *vacAm1s1i1* with 0.88%. Finally, *vacA* genotypes *m2s2i2* and *m1s2* were least frequent, with 0.44% (1/228) (Table 2).

Table 2. Prevalence of *Helicobacter pylori* genotypes typed.

Gene	Genotype	Prevalence (%)
cagA	*cagA+*	46 (20.2)
	cagA−	182 (79.8)
vacA	*vacA+*	154 (67.54)
	vacA−	74 (32.46
vacA+	*s1m1*	8 (3.51)
	s1m1I1	2 (0.88)
	s1m1i2	0 (0)
	s1m2	11 (4.82)
	s1m2i1	5 (2.19)
	s1m2i2	3 (1.31)
	m1m2	0 (0)
	s2m1	1 (0.44)
	s2m1i1	0 (0)
	s2m1i2	0 (0)
	s2m2	3 (1.31)
	s2m2i1	00 (0)
	s2m2i2	1 (0.44)
babA2	***babA2+***	22 (9.6)
	babA2−	206 (90.4)

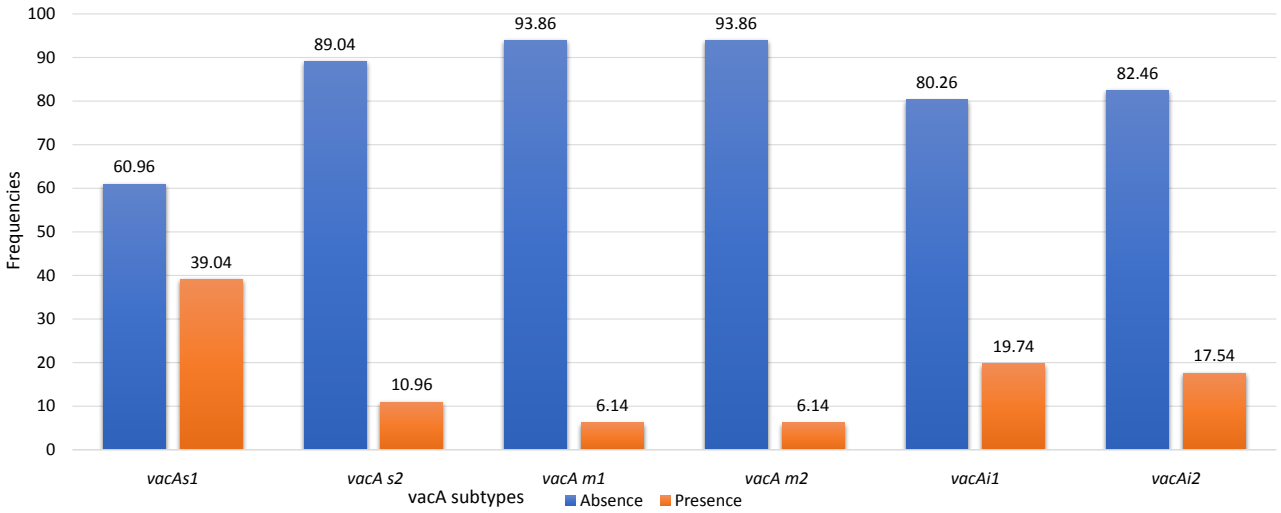

Figure 1. *Helicobacter pylori vacA* subtypes frequencies in our study population.

3.2. Relation among *cagA*, *babA2* and *vacA* Subtypes *s1*, *s2*, *m1*, *m2*, *i1* and *i2*

Table 3 shows the relation among *cagA*, *babA2*, and *vacA* subtypes. We noted that *vacAm2* and *vacAs1* were highly linked with a p-value = 0.004, while *babA2* was linked to *vacAs2* (p = 0.027). Additionally, *babA2* and *vacAm2* were significantly associated, with p = 0.045 (Table 3).

3.3. Relation between Socio-Demographical, Behavioral Conditions and *H. pylori* Virulence Genes *cagA*, *babA2*, and *vacA* Subtypes Carriage

Helicobacter pylori virulence genotypes association with some socio-demographical and behavioral factors was determined in Table 4. Of the collected information, only the number of handwashing per day was associated with *H. pylori* infection, highlighted by the presence of the rRNA16s gene, and was statistically significant (p-value = 0.018). Age was associated with *vacA* subtypes s2 and m2, and the p-values were 0.003 and 0.015, respectively, while sex and area of residency were not associated with the virulence genes in this study. The type of profession was associated with the *vacAi1* (p-value ≤ 0.001). The number of handwashing per day less than or over three (3) was associated with *vacAi2* (p-value = 0.004). The consumption of fresh products, such as fresh milk, fruits, and raw vegetables, was the most associated factor with the *vacA* subtypes: *vacAm1*, *vacAm2*, *vacAi1*, and *vacAi2* with p-values of 0.040, 0.008, 0.0001, and 0.012, respectively. Alcohol consumption was linked to the *babA2*, and the p-value was 0.013. The type of water source (running water or not) was associated with the virulence genotypes *cagA* (p ≤ 0.0001), *vacAi1* (p ≤ 0.003), and *vacAi2* (p ≤ 0.007). The number of persons per household was associated with *vacAs1* and *vacAi2*; p-values were 0.033 and 0.0001, respectively. Taking the meals alone or in a group was not associated with a virulence genotype.

Table 3. Relation among *Helicobacter pylori* virulence genes alleles studied.

	vacAs1	*vacAs2*	*cagA*	*babA2*	*vacA m1*	*vacAm2*	*vacAi1*	*vacAi2*
vacAs1	-	p = 0.449; p Cramer V = 0.330	p = 0.854; p Cramer V = 0.724	p = 0.072; p Cramer V = 0.064	p = 00.25; p Cramer V = 0.152	**p = 0.004; p Cramer V = 0.002**	p = 1; p Cramer V = 0.882	p = 0.451; p Cramer V = 0.351
vacAs2	p = 0.449; p Cramer V = 0.330	-	p = 0.195; p Cramer V = 0.118	**p = 0.027; p Cramer V = 0.010**	p = 0.975; p Cramer V = 0.637	p = 0.394; p Cramer V = 0.196	p = 0.195; p Cramer V = 0.118	p = 1; p Cramer V = 0.830
cagA	p = 0.854; p Cramer V = 0.724	p = 0.195; p Cramer V = 0.118	-	p = 0.249; p Cramer V = 0.152	p = 0.642; p Cramer V = 0.419	p = 0.823; p Cramer V = 0.571	p = 1; p Cramer V = 0.974	p = 0.496; p Cramer V = 0.369
babA2	p = 0.072; p Cramer V = 0.064	**p = 0.027; p Cramer V = 0.010**	p = 0.249; p Cramer V = 0.152	-	p = 0.4 27; p Cramer V = 0.207	**p = 0.045; p Cramer V = 0.013**	p = 0.635; p Cramer V = 0.449	p = 0.423; p Cramer V = 0.273
vacAm1	p = 0.250; p Cramer V = 0.152	p = 0.975; p Cramer V = 0.637	p = 0.642; p Cramer V = 0.419	p = 0.427; p Cramer V = 0.207	-	p = 0.679; p Cramer V = 0.323	p = 0.610; p Cramer V = 0.0391	p = 1; p Cramer V = 0.741
vacAm2	**p = 0.004; p Cramer V = 0.002**	p = 0.394; p Cramer V = 0.196	p = 0.823; p Cramer V = 0.571	**p = 0.045; p Cramer V = 0.013**	p = 0.679; p Cramer V = 0.323	-	p = 0.229; p Cramer V = 0.121	p = 0.975; p Cramer V = 0.693
vacAi1	p = 1; Cramer V = 0.882	p = 0.195; p Cramer V = 0.118	p = 1; p Cramer V = 0.974	p = 0.635; p Cramer V = 0.449	p = 0.610; p Cramer V = 0.0391	p = 0.229; p Cramer V = 0.121	-	p = 0.254; p Cramer V = 0.174
vacAi2	p = 0.451; p Cramer V = 0.351	p = 1; p Cramer V = 0.830	p = 0.496; p Cramer V = 0.369	p = 0.423; p Cramer V = 0.273	p = 1; p Cramer V = 0.741	p = 0.975; p Cramer V = 0.693	p = 0.254; p Cramer V = 0.174	-

3.4. Socio-Demographic and Behavioral Factors Associated with *H. pylori* Virulence Genes Carriage

Table 5 presents socio-demographic and behavioral factors associated with *H. pylori* virulence genes carriage. Handwashing equal to or less than three times per day increased significantly by almost four (4) and three (3) times the risk of having *vacAi2* genotype and *H. pylori* rRNA16s, with p-values = 0.013 and 0.020, respectively. Persons who did not consume fresh fruits or raw vegetables had eight (8) times the risk of having the *vacAi2* genotype, and it was statistically significant, p-value = 0.008. Furthermore, not drinking alcohol seems to reduce the risk of having *vacAi1* and *babA2* genotypes by 72% and 66%, and the p-values were 0.018 and 0.051, respectively. Persons who do not drink tap water (running water) have almost seven (7) times the risk of carrying the *cagA* virulence gene. Additionally, patients with more than four (4) people in their household had about two times the risk of *vacA* genotype s1, while it was a reduced risk by 67% for those carrying *vacAi2* genotype and p-value = 0.006.

4. Discussion

Many *H. pylori* virulence factors induce infection complications, leading to gastric cancer. In our context, biopsies and an *H. pylori* culture can be costly and

Table 4. Link between socio-demographical, behavioral conditions and *H. pylori* virulence genes, *cagA*, *babA2*, and *vacA* subtypes carriage.

(a)

Risk factors	Categories	rRNA16s			*vacAs1*			*vacAs2*			*vacAm1*			*vacAm2*		
		Negative	Positive	p-value	Negative	Positive	p-value	Negative	Positive	p-value	Negative	Positive	p-value	Negative	Positive	p-value
		N (%)	N (%)		N (%)	N (%)		N (%)	N (%)		N (%)	N (%)		N (%)	N (%)	
AGE	]20 - 40]	1 (0.4)	17 (6.8)	0.628	10 (4)	8 (3.20)	0.7	12 (4.80)	6 (2.40)	**0.003**	18 (7.20)	0 (0)	0.185	15 (6)	3 (1.20)	**0.015**
	]40 - 60]	15 (6)	125 (50)		88 (35.20)	52 (20.40)		131 (52.40)	9 (3.60)		129 (51.60)	11 (4.40)		137 (54.80)	3 (1.20)	
	>60	5 (2)	62 (24.8)		46 (18.40)	21 (8.40)		61 (24.40)	6 (2.40)		66 (26.40)	1 (0.40)		60 (24)	7 (2.80)	
	≤20	1 (0.4)	24 (9.6)		17 (6.80)	8 (3.20)		21 (8.40)	4 (1.60)		23 (9.20)	2 (0.80)		24 (9.60)	1 (0.40)	
SEX	F	13 (5.2)	133 (45.2)	0.945	95 (38)	51 (20.40)	0.794	134 (53.60)	12 (4.80)	0.266	138 (55.20)	8 (3.20)	0.922	135 (54)	11 (4.40)	0.115
	M	9 (3.6)	95 (38)		66 (26.40)	38 (15.20)		91 (36.40)	12 (4.80)		98 (39.20)	6 (2.40)		101 (40.40)	3 (1.20)	
Profession	Informal Sector	16 (6.4)	137 (54.8)	0.361	99 (39.60)	54 (21.60)	0.941	136 (54.40)	17 (6.80)	0.756	147 (58.80)	6 (2.40)	0.265	146 (56.40)	7 (2.80)	0.273
	Student	5 (2)	58 (23.2)		41 (16.40)	22 (8.80)		58 (23.20)	5 (2)		57 (22.80)	6 (2.40)		57 (22.80)	6 (2.40)	
	Civil servant	1 (0.4)	33 (13.2)		21 (8.40)	13 (5.20)		31 (12.40)	3 (1.20)		32 (12.80)	2 (0.80)		33 (13.20)	1 (0.40)	
Residence	Rural	0 (0)	8 (3.2)	0.372	5 (2)	3 (1.20)	0.909	6 (2.40)	2 (0.80)	0.151	8 (3.20)	0 (0)	0.484	7 (2.80)	1 (0.40)	0.388
	Urban	22 (8.8)	220 (88)		156 (62.40)	86 (34.40)		219 (87.60)	23 (9.20)		228 (91.20)	14 (5.60)		229 (91.60)	13 (5.20)	
Handwashing	≤3	10 (4)	160 (64)	**0.018**	107 (42.80)	63 (25.20)	0.483	153 (61.20)	17 (6.80)	1.000	159 (63.6)	11 (4.40)	0.383	160 (64)	10 (4)	0.777
	>3	22 (8.8)	68 (27.2)		54 (21.60)	26 (10.40)		72 (28.80)	8 (3.20)		77 (30.80)	3 (1.20)		76 (30.40)	4 (1.60)	
Fresh Product Consumption	No	13 (5.2)	157 (62.8)	.0348	111 (44.40)	59 (23.60)	0.667	153 (61.20)	17 (6.80)	1.000	157 (62.80)	13 (5.20)	**0.040**	156 (62.40)	14 (5.60)	**0.008**
	Yes	9 (3.6)	71 (28.4)		50 (20)	30 (12)		72 (28.80)	8 (3.20)		79 (31.60)	1 (0.40)		80 (32)	0 (0)	
Alcohol consumption	No	20 (8)	187 (74.8)	0.291	138 (55.20)	69 (27.60)	0.101	187 (74.80)	20 (8)	0.696	197 (78.80)	10 (4)	0.246	194 (77.60)	13 (5.20)	0.305
	Yes	2 (0.8)	41 (16.4)		23 (9.20)	20 (8)		38 (15.20)	5 (2)		39 (15.60)	4 (1.60)		42 (16.80)	1 (0.40)	
Treated water (tap water)	Other	3 (1.2)	40 (16)	0.643	23 (9.20)	20 (8)	0.101	37 (14.80)	6 (2.40)	0.342	43 (17.20)	0 (0)	0.079	43 (17.20)	0 (0)	0.079
	Tap water	19 (7.6)	188 (75.2)		138 (55.20)	69 (27.60)		188 (75.20)	19 (7.60)		193 (77.20)	14 (5.60)		193 (77.20)	14 (5.60)	
Number/ Household	>5	16 (6.4)	139 (55.6)	0.278	92 (36.80)	63 (25.20)	**0.033**	137 (54.80)	18 (7.20)	0.278	148 (59.20)	7 (2.80)	0.341	147 (58.80)	8 (3.20)	0.700
	≤5	6 (2.4)	89 (35.6)		69 (27.60)	26 (10.40)		88 (35.20)	7 (2.80)		88 (35.20)	7 (2.80)		89 (35.60)	6 (2.40)	
Meal alone / Meal in group	1	17 (6.8)	174 (69.6)	.920	120 (48)	71 (28.40)	0.350	169 (67.60)	22 (8.80)	0.150	178 (71.20)	13 (5.20)	0.136	179 (71.60)	12 (4.80)	0.398
	>1	5 (2)	54 (21.6)		41 (16.40)	18 (7.20)		56 (22.40)	3 (1.20)		58 (23.20)	1 (0.40)		57 (22.80)	2 (0.80)	

(b)

Risk factors	Categories	*vacAi1*			*vacAi2*			*cagA*			*babA2*		
		Negative	Positive	p-value	Negative	Positive	p-value	Negative	Positive	p-value	Negative	Positive	p-value
		N (%)	N (%)		N (%)	N (%)		N (%)	N (%)		N (%)	N (%)	
AGE	]20 - 40]	13 (5.20)	5 (2)	0.501	18 (7.20)	0 (0)	0.269	16 (6.40)	2 (0.80)	0.858	16 (6.40)	2 (0.80)	0.914
	]40 - 60]	113 (45.20)	27 (10.80)		117 (46.80)	23 (9.20)		113 (45.20)	27 (10.80)		129 (51.60)	11 (4.40)	
	>60	57 (22.80)	10 (4)		55 (22)	12 (4.80)		55 (22)	12 (4.80)		60 (24)	7 (2.80)	
	≤20	22 (8.80)	3 (1.20)		20 (8)	5 (2)		20 (8)	5 (2)		23 (9.20)	2 (0.80)	
SEX	F	120 (48)	25 (10)	0.925	125 (50)	21 (8.40)	0.409	121 (48.40)	25 (10)	0.537	132 (52.80)	14 (5.60)	0.602
	M	85 (34)	19 (7.60)		85 (35)	19 (7.60)		83 (33.20)	21 (8.40)		96 (38.40)	8 (3.20)	
Profession	Informal Sector	138 (55.20)	15 (6)	**≤0.001**	135 (54)	18 (7.20)	0.066	122 (48.80)	31 (12.40)	0.500	139 (55.60)	14 (5.60)	0.648
	Student	40 (16)	23 (9.20)		48 (19.20)	7 (2.80)		52 (20.80)	11 (4.40)		59 (23.60)	4 (1.60)	
	Civil servant	27 (10.80)	7 (2.80)		27 (10.80)	7 (2.80)		30 (12)	4 (1.60)		30 (12)	4 (1.60)	
Residence	Rural	8 (3.20)	0 (0)	0.178	6 (2.40)	2 (0.80)	0.480	5 (2)	3 (1.20)	0.157	8 (3.20)	0 (0)	0.372
	Urban	197 (78.80)	45 (18)		204 (81.60)	38 (15.20)		199 (79.60)	43 (17.20)		220 (80)	22 (8.80)	
Handwashing	≤3	135 (54)	35 (14)	0.120	135 (54)	35 (14)	**0.004**	140 (56)	30 (12)	0.654	155 (62)	15 (6)	0.985
	>3	70 (28)	10 (4)		75 (30)	5 (2)		64 (25.60)	16 (6.40)		73 (29.20)	7 (2.80)	
Fresh Product Consumption	No	127 (50.80)	43 (17.20)	**≤0.001**	136 (54.40)	34 (13.60)	**0.012**	142 (56.80)	28 (11.20)	0.251	157 (62.80)	13 (5.20)	0.348
	Yes	78 (31.20)	2 (0.80)		74 (29.60)	6 (2.40)		62 (24.80)	18 (7.20)		71 (28.40)	9 (3.60)	
Alcohol consumption	No	172 (68.80)	35 (14)	0.324	173 (69.20)	34 (13.60)	0.687	169 (67.60)	38 (15.20)	0.970	193 (77.20)	14 (5.60)	**0.013**
	Yes	33 (13.20)	10 (4)		37 (14.80)	6 (2.40)		35 (14)	8 (3.20)		35 (14)	8 (3.20)	
Treated water (tap water)	Other	42 (16.80)	1 (0.40)	**0.003**	42 (16.80)	1 (0.40)	**0.007**	25 (10)	18 (7.20)	≤0.001	36 (14.40)	7 (2.80)	0.057
	Tap water	163 (65.20)	44 (17.60)		168 (67.20)	39 (15.60)		179 (71.60)	28 (11.20)		192 (76.80)	15 (6)	
Number/ Household	>5	130 (52)	25 (10)	0.325	140 (56)	15 (6)	**≤0.001**	122 (48.80)	33 (13.20)	0.132	141 (56.40)	14 (5.60)	0.868
	≤5	75 (30)	20 (8)		70 (28)	25 (10)		82 (32.80)	13 (5.20)		87 (34.80)	8 (3.20)	
Meal alone / Meal in group	1	156 (62.40)	35 (14)	0.810	161 (64.40)	30 (12)	0.820	155 (62)	36 (14.40)	0.742	177 (70.80)	14 (5.60)	0.140
	>1	49 (19.60)	10 (4)		49 (19.60)	10 (4)		49 (19.60)	10 (4)		51 (20.40)	8 (3.20)	

challenging. Therefore, in this study, we searched for *Helicobacter pylori* rRNA16s and selected virulence factors in stool samples. The overall *Helicobacter pylori* frequency found in our study was 91.3% which is close to that of Werme *et al.*, in 2015 or Serme *et al.*, in 2016, who found respectively 91.43%, and 87.21% in Burkina Faso [3] [5]. These frequencies are similar to the 97% reported in the Gambia [21], 93.1% in Congo [22], 75% in Rwanda [23], while it was 69.9% in Morroco [24], and 50% in South Africa [25], although the studies were not done on the same type of samples. In the literature, *Helicobacter pylori* are transmitted early in life, especially in sub-Saharan Africa, explaining this high prevalence [4]. The early infection of *H. pylori* may be why the rates of gastric cancer are low in Africa. This high transmission of *Helicobacter pylori* is found by many studies to be probably from person to person, as in fecal-oral, gastric-oral, oral-oral, or through contaminated food and water [26] [27].

Table 5. Socio-demographic and behavioral factors associated with *H. pylori* virulence genes carriage.

(a)

Risk factors	Categories	*vacAs1*		*vacAs2*		*vacAm1*		*vacAm2*		rRNA16s	
		OR	p-value	OR	p-value	OR	p-value	OR	p-value	OR	p-value
AGE	]20 - 40]	1.911	0.328	3.541	0.117	≤0.001	0.998	4.168	0.251	0.560	0.695
	]40 - 60]	1.386	0.501	0.383	0.164	1.076	0.931	0.521	0.592	0.325	0.298
	>60	1.088	0.873	0.565	0.442	0.204	0.217	3.485	0.276	0.475	0.518
	≤20						Ref				
Sex	F	0.895	0.694	0.434	0.077	0.931	0.906	2.927	0.146	0.866	0.766
	M						Ref				
Profession	Informal Sector	0.951	0.904	1.186	0.820	0.801	0.807	1.271	0.847	0.276	0.239
	Student	1.053	0.915	0.823	0.824	1.403	0.719	1.976	0.596	0.368	0.400
	Civil servant						Ref				
Residence	Rural	0.950	0.949	2.719	0.337	≤0.001	0.999	6.139	0.227	1.510^{6}	0.999
	Urban						Ref				
Handwashing	≤3	1.614	0.124	1.129	0.813	2.082	0.330	0.964	0.959	**3.065**	**0.020**
	>3						Ref				
Fresh Product Consumption	No	0.500	0.993	1.262	0.709	3.347	0.274	6.110^{6}	0.996	2.083	0.192
	Yes						Ref				
Alcohol consumption	No	1.003	0.068	0.711	0.587	0.296	0.089	1.468	0.765	0.381	0.240
	Yes						Ref				
Treated water (tap water)	No	1.824	0.164	2.141	0.292	≤0.001	0.997	0.000	0.997	2.135	0.312
	Yes						Ref				
Number/ Household	>5	**1.804**	**0.047**	1.212	0.704	0.556	0.340	1.095	0.884	0.631	0.387
	≤5						Ref				
Meal alone/ Meal in group	1	1.600	0.185	3.274	0.105	3.502	0.266	0.959	0.962	0.915	0.880
	>1						Ref				

(b)

Risk factors	Categories	*cagA*		*vacAi1*		*vacAi2*		*babA2*	
		OR	p-value	OR	p-value	OR	p-value	OR	p-value
AGE	]20 - 40]	0.520	0.498	2.750	0.256	≤0.001	0.998	1.494	0.709
	]40 - 60]	1.039	0.948	1.674	0.465	0.620	0.437	0.764	0.748
	>60	0.854	0.804	1.344	0.698	0.814	0.755	1.035	0.968
	≤20					Ref			
Sex	F	0.775	0.470	1.115	0.780	0.841	0.660	1.157	0.764
	M					Ref			
Profession	Informal Sector	1.660	0.404	0.565	0.317	0.538	0.265	0.775	0.698
	Student	1.669	0.455	2.372	0.142	1.110	0.861	0.707	0.669
	Civil servant					Ref			
Residence	Rural	1.671	0.535	≤0.001	0.999	7.047	0.089	≤0.001	0.999
	Urban					Ref			
Handwashing	≤3	1.028	0.943	2.325	0.068	**3.914**	**0.013**	1.506	0.451
	>3					Ref			
Fresh Product Consumption	No	1.596	0.357	**8.154**	**0.008**	2.304	0.130	1.146	0.820
	Yes					Ref			
Alcohol consumption	No	1.119	0.819	**0.285**	**0.018**	0.627	0.404	**0.342**	**0.051**
	Yes					Ref			
Treated water (tap water)	No	**6.812**	**≤0.001**	0.208	0.184	0.131	0.090	2.540	0.148
	Yes					Ref			
Number/ Household	>5	1.325	0.467	0.849	0.666	**0.336**	**0.006**	0.999	0.998
	≤5					Ref			
Meal alone / Meal in group	1	1.702	0.257	0.541	0.201	0.648	0.357	0.604	0.340
	>1					Ref			

OR: Odds Ratio.

This study reports a strong association between sanitary habits and *Helicobacter pylori*'s virulence factors typed. Handwashing increased by three (3) times the risk of having *Helicobacter pylori* infection, while it was the "number of people in the household the subject grew up with" that was a risk factor in the studies of Smith *et al.*, in Nigeria [28], and Belay *et al.*, in Ethiopia [29]. The fact that persons did not drink tap water increased by six times their risk of carrying the virulence gene *vacA* when infected by *Helicobacter pylori*. However, in Santibanez *et al.*, study, *cagA* was related to active tobacco smoking in Spain [30]. Not drinking alcohol seems protective of carrying the *babA2* virulence gene within our research. Furthermore, we report here that *vacAs1*, *vacAi1*, and *vacAi2* are significantly associated with the number of people in a household, consumption of fresh products, and the number of times a person washes their hand per day, respectively.

Helicobacter pylori's pathogenicity island *cagA* is an oncoprotein due to nu-

merous associations with gastric cancer [9]. We report *cagA* with a frequency of 20.19% for our study population. This prevalence is surprisingly low compared to that of 74.8% reported in Ghana [31], 73.3% in Senegal [32], 52% reported in Gambia [21], 42.3% in Morroco [24], and 96.4% in Nigeria [33]. *BabA2*, another virulence gene associated with gastric epithelial cell adherence, has very few studies in sub-Saharan Africa. Here we report a prevalence of 9.65%, which is very low compared to the 83.3% reported in Cuba [34] and 94.6% reported in Iran [35]. Furthermore, *vacA* or vacuolating cytotoxin is involved in the progression of gastroduodenal diseases. The gene has a toxigenic effect as it binds to the eukaryotic lipid sphingomyelin receptor; it then targets mitochondria, induces apoptosis, and makes large extracellular vacuoles.

VacA genes have polymorphisms and are structured mainly into signal, intermediate, middle, and deleted regions; its frequency is 67.54%. The *vacAs1* allele was the most represented in our study population, similar to the studies from Senegal, Ghana, The Gambia, and South Africa [21] [25] [31] [32]. The allelic combination *vacAs1/m1* is the most virulent, whereas *s1/m2*, *s2m1*, and *s2m2* genotypes show low to no pathogenicity [12]. We report in our study the presence of *s1m2* (4.82%), s*1m1* (3.51%), *s1m2i1* (2.19%), *s2m2* (1.31%), *s1m1i1* (0.88%), *s2m1* (0.44%), and *s2m2i2* (0.44%), similarly to Rhead *et al.*, [36]. Overall, the frequencies of *cagA*, *vacA*, *vacA* subtypes, and *babA2* were low than those reported by Archampong *et al.*, in Ghana, Breurec *et al.*, in Senegal, and Idowu *et al.*, in South Africa [25] [31] [32]. These differences could be due to the type of the study population, the type of sample used, and the strains of *H. Pylori* present.

The women were the most represented group in our study population; however, the Helicobacter pylori infection rate was higher among men (78.3%) than women (67.4%), which was insignificant. The gender difference in *H. pylori* infection was also reported in previous studies by Compaore *et al.*, Replogle *et al.*, and de Martel *et al.*, and the immune system response can partially explain this difference, as women might have protective immunity against *H. pylori* [37] [38] [39]. Studies imply that immune response differs between men and women [40]. Biologically, estrogen stimulates immune responses, while testosterone is immunosuppressive [41]. Many virulence genes of *H. pylori* have been associated with peptic ulcer, duodenal and gastric cancer. However, due to incomplete patient data, this study did not use samples from known gastric cancer patients or gastroduodenal diseases. Our research shows the presence of several *H. pylori* virulence factors in stools, but the link between these factors and *Helicobacter pylori*-related diseases is yet to be thoroughly investigated. This study is a stepstone that allows clinicians and researchers to know which subtype of *vacA* is present in our context. This information can be used in research to improve the eradication treatment in a context of antibiotic resistance. It may also help clinicians predict patients at risk for gastric cancer due to their *VacA* and *CagA* virulence gene profiles.

5. Conclusion

The present study shows the presence of *Helicobacter pylori* virulence genes *cagA*, *vacA*, *vacA* subtypes, and *babA2* in stool samples by polymerase chain reaction method in Burkin Faso. Additionally, the strong association between sanitary habits and virulence factors typed depicts the composite interaction between ecological factors, gastric mucosa, and bacteria. Therefore, synergic action of these factors needs to be considered when aiming for the bacteria's eradication and gastric pathology cure.

Authors' Contributions

TRC and KT conceived and designed the experiments and wrote the manuscript; TRC, KT, NIC, LT, SZ, STS, DK, DS, YAS, 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.

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.

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Conflicts of Interest

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

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