African Helicobacter & Microbiota Study Group

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Detection of *Helicobacter pylori* genotypes, *cagA*, *vacA m1* and *vacA m2* and their correlation with gastric histopathology: a cross sectional study in Yaoundé, Cameroon.

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- OBJECTIVES
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I. INTRODUCTION (1/3)

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- *Helicobacter pylori* (*H. pylori*) is a micro-aerophilic Gram-negative bacteria that colonizes the gastric mucous layer and the epithelial lining of the stomach (Yu *et al*., 2017).
- It is global public health concern with 4.4.billion people infected worldwide (Hooi *et al*., 2017).
- Globally, the prevalence of *H. pylori* is 50.8% in developing countries and 70.1% in Africa (Hooi *et al*., 2017).

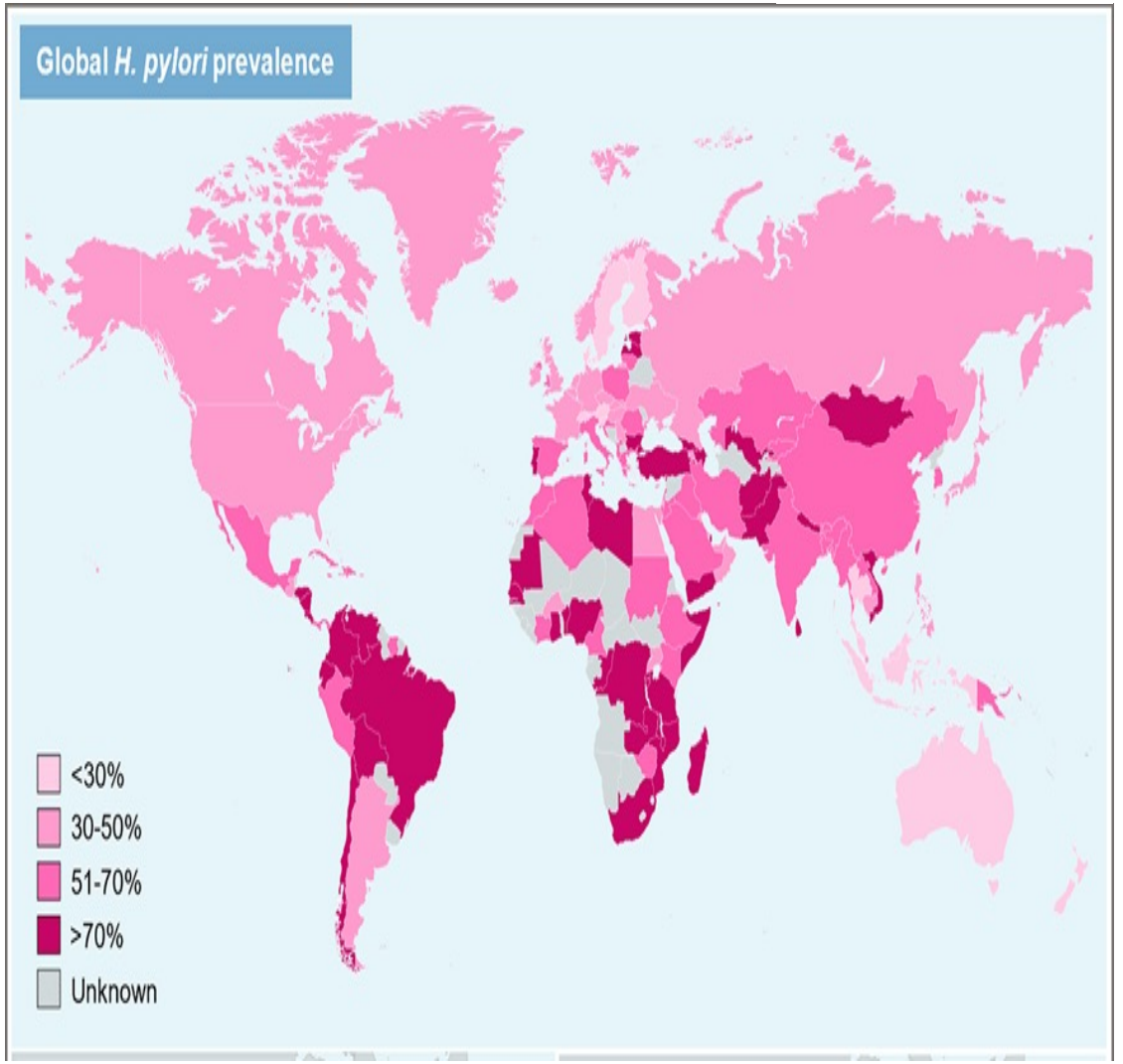

Figure 1: global *H. Pylori* prevalence.

I. INTRODUCTION (2/3)

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- To date, 20 strains of *H. pylori* have been recognized and evidence has shown their implication in many diseases including duodenal ulcers, gastric ulcers, adenocarcinoma of the distal stomach, and mucosa-associated lymphoid tissue (MALT) and lymphoma (Yu *et al*., 2017).
- The virulence of *H. pylori* is correlated with several pathogenicity genes *(cagA, vacA, babA2, dupA, iceA, and oipA)*, whose proteins take part in colonization procedures, immune response evasion, and the presence of these genes has been considered to be a predictor of severe clinical consequences (Sharndama *et al*., 2022).

I. INTRODUCTION (3/3)

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- The Sydney grading system for chronic gastritis and, its updated Houston version, are the commonly used nomenclature for gastritis. This system categorizes gastritis according to intensity of mononuclear inflammatory cellular infiltrates, polymorph activity, atrophy, intestinal metaplasia, and *H. pylori* density into mild, moderate and severe categories (Dixon *et al.*,1999).

- In the Operative Link for Gastritis Assessment (OLGA) staging system, an atrophybased staging system would provide implications for the prognosis and, possibly, the management of patients (OLGA Group,2005).

II. OBJECTIVES (1/1)

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II.1. Specific objectives

- To determine the prevalence of *H. pylori* in symptomatic patients using the *glmM* gene and histopathology analysis.
- To determine the prevalence of *H. pylori* genotypes *cag A, vacA m1,* and *vacA m2* virulence genes.
- To investigate the correlation of *H. pylori* genotypes with gastric histopathology findings in relation to the updated Sydney and OLGA staging system.

III. METHODOLOGY (1/7)

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III.1. Type of study: Quantitative, prospective, cross-sectional and descriptive study

III.2. Period: From october 2015 to April 2016

III.3. Study site: *Centre Medical la Cathedral,* General Hospital of Yaounde and Laboratory for Public Health Research Biotechnologies Yaounde- Cameroon.

III.4. Population: Patients of both sexes, referred for endoscopy.

III.5. Sampling method: Consecutive using structured questionnaire.

III.6. Sample size: One hundred and forty-seven (147) using this formula: $n = Z^2 pq/d^2$

III. METHODS (2/7)

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III.7. Inclusion criteria

- Patient who had not received treatment with broad spectrum antibiotics, non-steroidal anti-inflammatory drugs or proton pump inhibitors in the previous 3 months;
- Patient who did not have a history of dysphagia, gastric surgery or upper gastro intestinal bleeding;
- Patient who have signed the consent form were enrolled.

III. METHODS (3/7)

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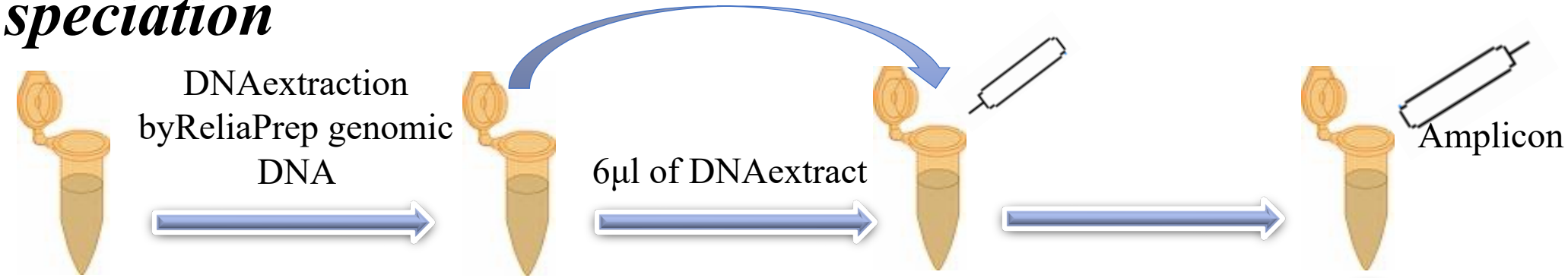
III.8. Collection of Samples

- Four gastric biopsy specimens (3antrums and 3corpus) were collected from each of the patients, by a gastroenterologist during endoscopy using an Olympus GIF – Q30.

III.9. Sample Processing: Two biopsies were introduced into a container with 2ml of TRIS EDTA buffer for detection of the *glmM, cagA, vacA m1* and *vacA m2* genes using PCR,Two other biopsies vere introduced into a container with formaldehyde 10% for histological analysis.

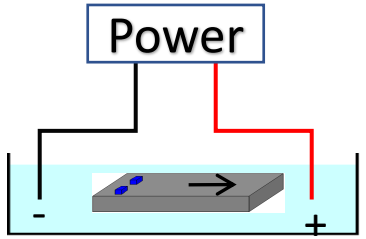
III. METHODS (4/7)

III.10DNA extraction, *glmM, cagA, vacA m1* and *vacA m2genes speciation*



Master mix: 12.5 µl Premix Mix
NFW= **3.5µl**
One Taq hot start mix 2mM=0.05u/µl
Primers:(F/R):
[*glmM, cagA, vacA m1* and *vacA m2* genes]=**1.5µl** each

10µl amplicon for electrophoresis



2% Agarose gel

Visualization under UV light and gel photographed

III. METHODS (5/7)

III.11 . Histological examinations

- The two biopsies in the tube containing 2mL of Formaldehyde at 10% was used for histology analysis.

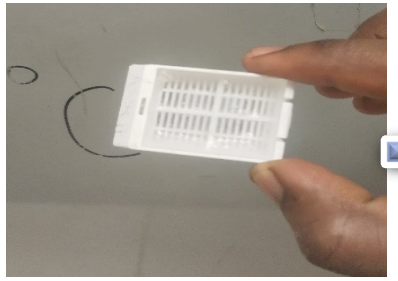


Fig:2 ACP cassette

Fig;3 Paraffin waxcontainers

Fig:4 Coolingsystem

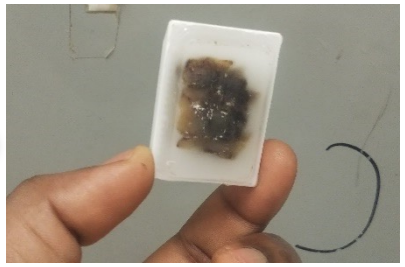


Fig:5 Paraffin wax block with histological specimen

Fig:6 Microtome for cutting

Fig:7 Hematoxylin and eosin staining tanks

III. METHODS (6/7)

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III. Statistical Analysis

•The data were entered, cleaned, edited and coded, using Excel database, and then exported to SPSS v. 20.0 for analysis. The chi-square test and Fisher's exact test were used to assess the relationship between individual genotypes and the histopathology results. Logistic regression analysis was used to measure the association between the OLGA staging system and the frequencies of virulence genes *cagA, vacAm1* and *vacAm2*. The differences were considered to be statistically significant when the p-value obtained was less than 0.05.

III. METHODS (7/7)

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III. Ethical Considerations

- This study was conducted in accordance with the Helsinki Declaration and the national laws and regulations. The study was approved by the Centre Regional Ethics Committee for Human Health Research (CE 032 N°/CRERSHC/2015).Administrative authorizations were obtained from the Regional Delegation of Public Health for the Center Region, and the Director of the *Centre Medical La Cathedral*. A written informed consent from adult, parental or guardian for children (age under 21years old) was sought from patients after details of the study were explained to them. Emphasis was laid on the voluntary nature of participation and that they could withdraw at any time without any explanation. Confidentiality was ensured by the use of unique identification codes attributed to each study participant.

IV. RESULTS (1/5)

IV.1. Prevalence of *H. pylori* infection and Sociodemographic characteristic

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Table 1: Prevalence of *H pylori* infection associated with socio-demographique factors

Variables	Category	Positive/tested	Prevalence (%)	p-value
Gender	Female	28/88	31.8	0.49
	Male	22/59	37.3	
Age (years)	≤30	8/23	34.8	0.26
	[30-50]	26/64	40.6	
	≥50	16/60	26.7	
Region of origin	Africa (excluding Cameroon)	4/11	36.4	
	Center region	14/63	46.03	0.034*
	Far north region	3/11	27.3	
	West region	29/62	46.8	
Level of education	Primary	19/52	38.5	
	Secondary	14/41	28.8	0.001*
	Univresity	19/54	38.0	
Occupations	Primary sector	14/42	28.00	
	Secondary sector	08/26	16.00	0.004*
	Tertiary sector	28/79	56.00	
Alcohol consumption	Yes	30/83	37.3	0.0001*
	No	20/64	29.7	
Tobacco smooking	Yes	6/18	33.3	0.94
	No	44/129	34.1	
Family size	≥3persons/room	06/37	16.21	0.94
	<3 pesons/room	44/110	40.00	

The frequency of *glmM* genes was **34.01%** (50/147) and **85.71%** (129/147) for Histopathological diagnostic

IV. RESULTS (2/5)

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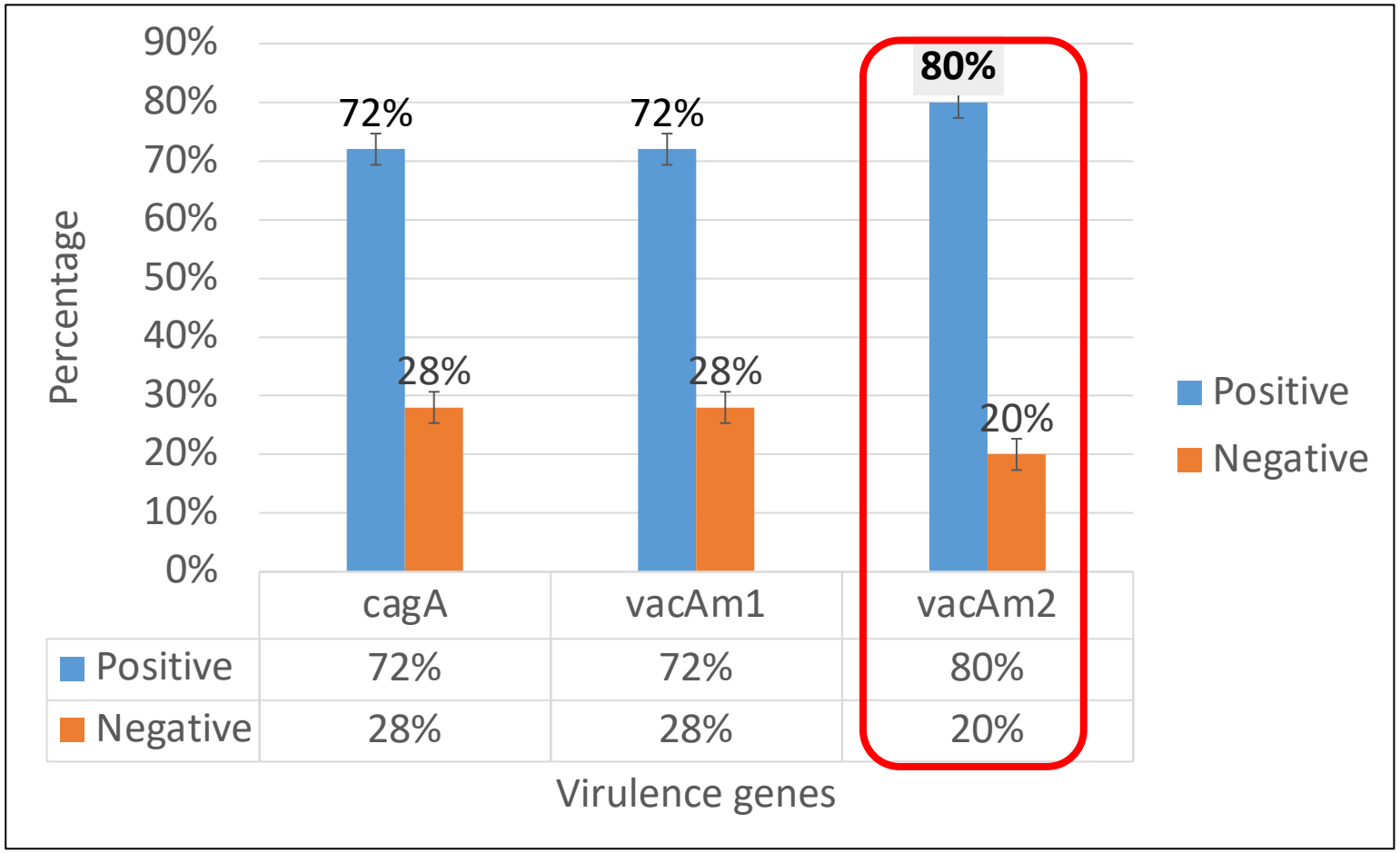
IV.2. Prevalence of *cagA, vacAm1, and vacAm2* genotypes



The *cagA* gene was present in **72%** (n=36)

The *vacA* m1 and m2 were present in **72%** (n=36) and **80%** (n=40) respectively.

Figure 8: *H. pylori* prevalence virulence genes in study population.

IV. RESULTS (3/5)

IV. Association between clinical outcomes and virulence genes in 50 *H. pylori* strains.

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Table2 Association between clinical outcomes and virulence genes in 50 *H. pylori* strains.

Variables	Total N=50	CagA + n=36 (%)	OR, 95%CI	p	vacAm1+ n=36 (%)	OR, 95%CI	p	vacAm2+ n=40 (%)	OR, 95%CI	p
ATCD gastric	1	1 (100)	-	0.62	1 (100)	-	0.62	1 (100)	-	0.61
Balance HTP	2	2 (100)	-	0.92	1 (50.0)	0.37 (0.02-0.38)	0.92	2 (100)	-	0.92
Left hypochondrial pain	0	0	-	-	0	-	-	0	-	-
Dyspepsia	1	1 (100)	-	0.62	1 (100)	-	0.62	1 (100)	-	0.62
Dysphagia	2	1 (50.0)	0.37 (0.02-0.38)	0.92	2 (100)	-	0.92	1 (50.0)	0.23 (0.01-4.05)	0.27
Epigastralgia	32	20 (62.5)	0.20 (0.04-1.06)	0.04	24 (75.0)	1.5 (0.43-5.31)	0.52	24 (75.0)	0.37 (0.07-1.99)	0.23
Chronic epigastralgia	7	6 (85.71)	2.60 (0.28-23.81)	0.62	5 (71.43)	0.96 (0.16-5.68)	0.97	7 (100)	-	0.53
Gastralgia	1	1 (100)	-	0.62	0	-	-	1 (100)	-	0.62
Low digestive hemorrhage	0	0	-	-	0	-	-	0	-	-
High digestive hemorrhage	2	2 (100)	-	0.62	2 (100)			2 (100)	-	0.47
Precordialgia	0	0	-	-	0	-	-	0	-	-
Heartburn	1	1 (100)	-	0.62	0	-	-	1 (100)	-	0.62
RGO	1	1 (100)	-	0.62	0	-	-	0	-	-

Epigastralgia had a significant correlation with *cagA* (p= 0.04, OR=0.20 95% CI 0.04-1.06).

IV. RESULTS AND DISCUSSION (4/5)

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IV.3. Prevalence of *cagA*, *vacAm1*, *vacAm2* genotypes with Sydney system

Table 3: Prevalence of virulence genes cagA, vacAm1 and vacAm2 associated with Sydney system

	cagA		*vacAm1*		*vacAm2*	
Variables	Positive (%)	p-value	Positive (%)	p-value	Positive (%)	p-value
Differenciation A						
Dysphagia	5 (83.33)	0.86	6 (100.0)	0.44	5 (83.33)	0.72
High grade dysplasia	1 (100.0)		1 (100.0)		1 (100.0)	
Without atypia	30 (69.77)		29 (67.44)		34 (79.07)	
Differenciation F						
Without atypia	36 (72.00)		36 (72.00)		40 (80.00)	
Degree A						
Active moderate	5 (62.50)	0.96	7 (87.50)	0.75	7 (87.50)	0.96
Active severe	2 (100.0)		2 (100.0)		2 (100.0)	
Little active	29 (72.50)		27 (67.50)		31 (77.50)	
Degree F						
Active moderate	2 (28.57)	0.07	5 (71.43)	0.92	4 (57.14)	0.55
Active severe	2 (100.0)		2 (100.0)		2 (100.0)	
Little active	32 (78.05)		29 (70.73)		34 (82.93)	

For the Sydney staging system, Dysphagia and Litt active, had a significant correlati with the *cagA* gene (OR=2.096, 95% C 0.22-19,74) and (p=0.86, OR=4.44 95% CI 0.98-20.08) respectively ; *vacA* (p=0.74, OR=2.42 95% CI 0.48-12.11) with Little active ar *vacAm1*(OR=3.13 95% CI 0.34-28.10) with Active modera

IV. RESULTS (5/5)

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IV.4. Prevalence of virulence genes *cagA, vacAm1 and vacAm2* with OLGA staging system

Table 4: prevalence of virulence genes *cagA, vacAm1* and *vacAm2* with OLGA staging system

Variables	Total N=50	CagA + n=36 (%)	OR, 95%CI	p	vacm1+ n=36 (%)	OR, 95%CI	p	vacm2+	OR, 95%CI	p
Stage I	14	8 (57.14)	2.62 (0.70-9.80)	0.14	5 (35.71)	0.08 (0.02-0.38	0.001	9	0.60 (0.15-2.26)	0.44
Stage II	10	7 (70.0)	1.12 (0.24-5.16)	0.81	10 (100)	-	0.02	8	1.71 (0.31-9.29)	0.52
Stage III	13	10 (76.93)	0.70 (0.16-3.08)	0.64	9 (69.23)	0.83 (0.20-3.32)	0.79	10	1.41 (0.32-6.13)	0.64
Stage IV	13	11 (84.62)	0.37 (0.07-1.98)	0.23	12 (92.31)	6.50 (0.75-55.73)	0.05	13	-	0.02

Stage IV had a significant correlation with the *vacAm1* gene (p=0.05, OR=6.50 95% CI 0.75-55.73) with respect to the OLGA staging system.

V. CONCLUSIONS (1/1)

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The result of this study provide baseline data on *H. pylori* genotypes circulating in Yaoundé Cameroon ; this would be useful for prospective epidemiological and genetic investigation to better understand the diversity and control of *H pylori* related morbidity.

PERSPECTIVES (1/1)

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•A similar study with a large sample size needs to be carried out in other areas of Yaoundé and in the entire country, as well as the inclusion of healthy individuals as a control group in order to have a clear prevalence of *H pylori* infection.

• Sequence analysis of *H. pylori cagA, vacAm2, vacAm1* virulence genes.

ACKNOWLEDGEMENTS (1/1)

- **Nkoth AF[1,2], Kabeyene A[2], Njoya I[2], Mbacham WF[3], Ndip RN[1]**
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THANKS FOR YOUR KEEN ATTENTION