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ORIGINAL ARTICLE

Histomolecular profile of *Helicobacter pylori* strains circulating in Brazzaville (Congo)

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Abstract

Background and Aim: *Helicobacter pylori* (Hp) infection is a real public health problem in the Congo. We aimed study the histomolecular profile of Hp strains circulating in Brazzaville, Congo, in order to contribute to the improvement of Hp-infected patients in the country.

Methods: This was an analytical-transversal study carried out from January to November 2020 (i.e. a study period of 11 months) in the endoscopy centers of Brazzaville as well as the molecular biology and anatomopathology laboratories of Pointe-Noire and Oyo. It involved 100 symptomatic patients over the age of 18 referred for upper GI endoscopy. These patients underwent gastric biopsies for histopathological analysis according to the Sydney classification and molecular analysis using the real-time polymerase chain reaction (PCR) technique. The frequency of Hp infection was determined using real-time PCR. PCR was also used to identify the Hp strains and assess their tropism in the gastric mucosa. Digestive symptoms, endoscopic lesions, and histopathological lesions associated with HP infection were studied.

Results: The incidence of Hp infection was 91%, with a female predominance of 52.75% and an average age of 46.32 years. Endoscopy revealed normal mucosa (56.14%), ulcerated lesions (12.28%), and gastritis (22.81%) in infected patients. Histopathologically, the lesions were chronic atrophic gastritis (91%), with inflammatory activity (16.46%), intestinal metaplasia (16.46%), and adenocarcinoma (3.3%). Cag A strains were present in 85.71% of cases and had no preferential tropism in the gastric mucosa. Strains carrying the Cag A gene were present in severe and serious endoscopic and histopathological lesions.

Conclusion: The prevalence of Hp infection is 91% in the Brazzaville population. Cag A strains circulate in high proportions and are implicated in the occurrence of severe lesions of the gastric mucosa.

Introduction

Helicobacter pylori (Hp) is a gram-negative, spiral-staining bacterium, the leader of the *Helicobacter* genus. It specifically colonizes the gastric mucosa. Its pathogenicity is linked to the existence of several factors, the main ones being flagella (4–7), urease, adhesins, pathogenicity islands, and the Cag A protein.[1–4] Cag A, the effector protein, is the pathogenicity factor incriminated in the development of chronic atrophic gastritis, adenocarcinomas, and mucosa-assisted lymphoid tissue (MALT) lymphomas. In 1994, WHO declared Hp to be a class I carcinogen, making it the only bacterium with carcinogenic potential.[3]

Hp infection is a real concern for human health worldwide. It is the most widespread chronic bacterial infection, particularly in developing countries.[5] More than 50% of the world's population is thought to be infected, and in Africa the prevalence is between 60% and 91%.[6-10] In Congo, Ontsira Ngoyi *et al*. in 2015 estimated the frequency at 89% in adults.[11] Itoua-Ngaporo *et al*. in 2018 estimated that Hp was present in 79.6% of the population.[12]

The expression of the Cag A oncoprotein has enabled strains of Hp to be classified into two groups: those that carry the Cag A gene, and those that do not. The prevalence of CagA strains worldwide is 70%, rising to 90–95% in East Asia, particularly in South Korea, China, and Japan. According to Kidd *et al*.,[13] all South African strains carry this gene. The literature describes that strains carrying the Cag A gene are more pathogenic than those that do not express it.

The wild or mutated strains circulating in the Congo Basin are not as well known as their pathogenicity. The aim of this work is to improve the management of patients infected with Hp by studying the molecular biodiversity of the strains circulating in Brazzaville and their degree of pathogenicity.

Methods

This was an analytical and cross-sectional study from January to November 2020 (i.e. a period of 11 months). The study was conducted in Brazzaville and Pointe-Noire. For this study, upper digestive endoscopy and biopsy samples were taken in three endoscopy centers in Brazzaville, namely the Schnell Foundation Medical and Social Center, the OCH Gastroenterology Medical Center, and the Gastroenterology and Internal Medicine Department of the Brazzaville University Hospital. The histopathological study was carried out at the anatomo-pathology laboratory of the Edith Lucie Mbongo Odimba Hospital (HGELBO) in Oyo, and the molecular study was conducted at the Hugues Dieudonné Loemba Laboratory (HDL) of the Marie Madeleine Gombes Foundation (FMMG) in Pointe-Noire. The study population consisted of all patients who underwent esogastroduodenal endoscopy (EOGD), regardless of the indication, at one of the investigation centres during the study period. We included all consenting patients over 18 years of age, symptomatic patients, and those with histopathologically confirmed gastric cancer. Patients who had taken a proton pump inhibitor (PPI) and/or an antibiotic in the month prior to endoscopy, patients in whom EOGD was incomplete and biopsy was not possible, and patients in whom biopsies were inoperable were excluded from the study. Our sample size was 100 patients. Patients meeting the inclusion criteria were sampled consecutively until the estimated number was reached. The study received the approval of the Health Sciences Research Ethics Committee (No. 303/MRSIT/IRSSA/CERSSA).

Data were collected by the same investigator using a pre-established survey form. This was used to collect information on digestive symptoms as well as endoscopic and histopathological data. Upper GI endoscopy was performed using Fujinon (Fujifilm) video endoscopes at the Brazzaville University Hospital and Elvis Exera II (Olympus) GIF V2, GIFQ 145 at the other centers, as well as accessory equipment that had been carefully disinfected and sterilized prior to the examination in accordance with the recommendations of the Société Française d'Endoscopie Digestive (SFED). During the examination, samples were taken using a single-use biopsy forceps from two sites: the antral mucosa and the fundic mucosa. For each site, two biopsy fragments approximately 0.5 mm in diameter were taken. For each of the two sites, one fragment was used for histopathology and the other for molecular biology. The biopsy samples intended for the former were preserved and fixed in 10% buffered formalin, and those intended for the latter were cryopreserved (frozen at −32 °C).

The histopathological analysis of the biopsy samples was carried out in accordance with current procedure and took place in three consecutive phases: the pre-analytical phase, which consisted of preserving the integrity of the tissues and the Hp genes; the analytical phase, which consisted of microscopic study and analysis of the lesions; and the post-analytical phase, which consisted of transcribing the histopathological results onto the investigation form according to the elements of the Sydney classification.

The molecular study was carried out on fresh cryopreserved biopsy samples, and included DNA extraction and double amplification of Hp. The DNA was extracted using the ReliaPrep gDNA tissue Miniprep system from Promega, following the manufacturer's instructions. The second stage of the polymerase chain reaction (PCR) consisted of a double amplification of the microbial DNA. The first amplification allowed detection of the bacteria by amplifying the DNA polymerase beta subunit gene "rpoB," present in all Hp strains, using the Techne PrimePRO qPCR DNA detection Kit, *H. pylori*. All Hp-positive samples were subjected to a second amplification, which allowed the identification and determination of the strains by expression of the Cag A gene, using the Microbial DNA qPCR Multi-Assay *H. pylori* amplification kit (Qiagen Sciences, MD, USA).

The data were processed and analyzed using SPSS 25 and Microsoft Excel. Pearson's Chi-square tests were used for analysis.

Results

The molecular frequency of Hp infection was 91%. Our study population was predominantly female, and 52.75% of women were carriers of the bacterium. The mean age of the patients was 46.32 ± 15.20 years, and the peak infection (32.97%) was between 40 and 49 years of age (Fig. 1).

Out of a total of 100 patients included in our study, 87 presented with abdominal pain. Hp was found in 92.5% of patients presenting with epigastric pain, 65% of which was of the burning type (Table 1). All patients included in the study had undergone upper GI endoscopy. Endoscopy was normal in 70% of patients and revealed pathological mucosa in 30%. Endoscopy was indicated for epigastralgia in 93.1% of cases. In infected patients, endoscopy revealed normal mucosa (56.14%), ulcerated lesions (12.28%), and gastritis (22.81%). The bacterium was exclusive to acanthosis-type lesions (100%) and frosted lesions (100%). The results are shown in Table 2.

Histopathlogically, the presence of a lymphoplasmacytic infiltrate indicates chronic inflammation of the gastric mucosa, while the presence of neutrophils indicates inflammatory activity. We found 15 cases of chronic active gastritis, 18 cases of severe chronic atrophic gastritis, 15 cases of chronic gastritis with intestinal metaplasia, and 4 cases with gastric adenocarcinoma. However, there were no cases of lymphoma (Table 3).

Hp was present in all serious diseases of the gastric mucosa, that is active, moderate to severe atrophic chronic gastritis (GCA), chronic gastritis (GC) with a focus of metaplasia or dysplasia, and, above all, in adenocarcinomas. Carriage of the infection was 100% in severe GCA, GC with inflammatory activity, and in GCA with intestinal metaplasia. There was no significant difference between Hp infection and the histopathological lesions found (Table 3).

Normal aspects of the gastric mucosa corresponded for the most part to mild GCA, but also represented the nest of chronic atrophic gastritis with intestinal metaplasia. However, the

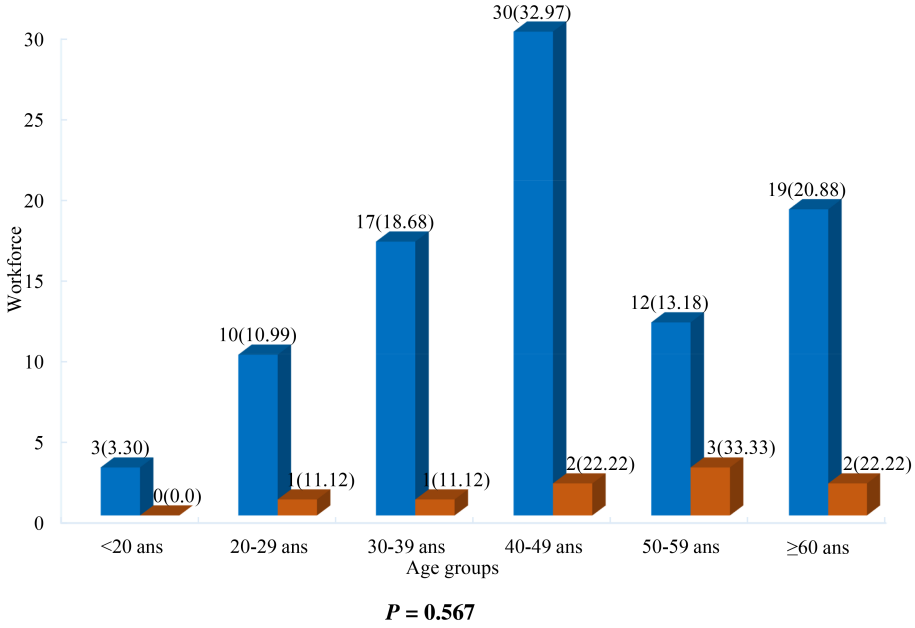

Figure 1 Distribution of infection according to age. (■), Positive.

Table 1 Distribution of infection according to type of pain

	PCR results		
	Positive ($n = 91$)	Negative ($n = 9$)	
Variables	*n* (%)	*n* (%)	*P*-value
Site of pain			
Epigastrium	74 (92.5)	8 (100)	0.4
Right hypochondrium	2 (2.5)	—	0.6
Broadcast	4 (5.0)	—	0.5
Type of pain			
Burn	52 (65)	3 (42.86)	0.2
Cramp	25 (31.25)	4 (57.14)	0.1
Vice	1 (1.25)	—	0.7
Not specified	2 (2.50)	—	0.6

PCR, polymerase chain reaction.

ulcerating-bourgeous and infiltrated lesions showed the macroscopic appearance of an adenocarcinoma with areas of intestinal metaplasia (Table 4).

Molecular amplification by expression of the Cag A gene isolates two types of strains: those expressing the CagA gene, and those not expressing it. Strains carrying the Cag A gene were found in 78 patients (i.e. a frequency of 85.71%). These strains were found simultaneously in both sites in 72 patients (Fig. 2). They are found almost exclusively in all endoscopic pathological aspects of the gastric mucosa (Table 5). Strains carrying the Cag A gene are incriminated in moderate to severe chronic atrophic gastritis with intestinal metaplasia and in adenocarcinomas (Table 6).

Discussion

Considered to be the only carcinogenic bacterium to date, Hp infection is a major public health concern in developing countries. The aim of this study was to investigate the molecular biodiversity of the strains circulating in Brazzaville and their pathogenicity.

Molecular biology was used to assess the carriage of Hp infection in the study population. Of the 100 patients in our study population, 91 were PCR positive (i.e. a frequency of 91%). This frequency was higher in women (52.75%). Aguemon *et al.*[7] in Benin and Bommelaer *et al.* in France also reported a predominance of the infection in females,[4,14] whereas Attaf *et al.* in

Table 2 Distribution of infection according to endoscopy results

	PCR results		
	Positive ($n = 91$)	Negative ($n = 9$)	
Variables	*n* (%)	*n* (%)	*P*-value
Endoscopic aspects of patients			
Normal mucosa	64 (56.14)	6 (37.5)	0.8
Ulcerated lesion	14 (12.28)	3 (18.75)	0.1
Budding lesion	3 (2.63)	2 (12.5)	**0.01**
Frosted lesion	1 (0.88)	—	0.7
Erosive aspect	12 (1.53)	1 (6.25)	0.8
Congestive aspect	14 (12.28)	1 (6.25)	0.7
Infiltrated appearance	1 (0.88)	2 (12.5)	**0.0003**
Acanthosis lesion	2 (1.75)	—	0.6
Other	3 (2.63)	1 (6.25)	0.2

Values in bold correspond to the variables of interest.
PCR, polymerase chain reaction.

Table 3 Distribution of infection according to histopathological findings

	PCR results		
	Positive ($n = 91$)	Negative ($n = 9$)	
Chronic gastritis with	*n* (%)	*n* (%)	*P*-value
Inflammatory activity	15 (16.48)	—	0.2
Glandular atrophy			
Slight	55 (60.44)	6 (6.50)	0.3
Moderate	18 (19.78)	—	
Severe	18 (19.78)	—	
Intestinal metaplasia	15 (16.48)	—	0.8
Dysplasia			
Low grade	1 (1.1)	—	0.1
High grade	2 (2.2)	—	
ADK	3 (3.3)	1 (1.1)	0.1
MALT lymphoma	—	—	

ADK, adenocarcinoma; PCR, polymerase chain reaction; MALT, mucosa-assisted lymphoid tissue.

Table 4 Correlation between endoscopic lesions and histology

	Chronic atrophic gastritis					
	Slight	Moderate	Severe	MI	ADK	
Variables	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*P*-value
Endoscopic aspects						
Normal mucosa	48 (78.7)	13 (72.2)	7 (38.9)	6 (40.0)	—	**0.005**
Ulcerated lesion	7 (53.8)	4 (80.0)	5 (45.5)	6 (66.7)	3 (100.0)	0.4
Budding lesion	2 (15.4)	—	2 (18.2)	1 (11.1)	3 (100.0)	0.7
Frosted lesion	1 (7.7)	—	—	—	—	0.4
Erosive aspect	8 (61.5)	1 (20.0)	4 (36.4)	2 (22.2)	—	0.1
Congestive aspect	8 (61.5)	1 (20.0)	6 (54.5)	3 (33. 3)	—	0.1
Infiltrated appearance	1 (7.7)	—	1 (9.1)	1 (11.1)	2 (66.7)	0.5
Acanthosis lesion	1 (7.7)	—	1 (9.1)	—	—	0.3
Other	—	1 (20.0)	2 (18.2)	1 (11.1)	2 (66.7)	0.9

Values in bold correspond to the variables of interest.

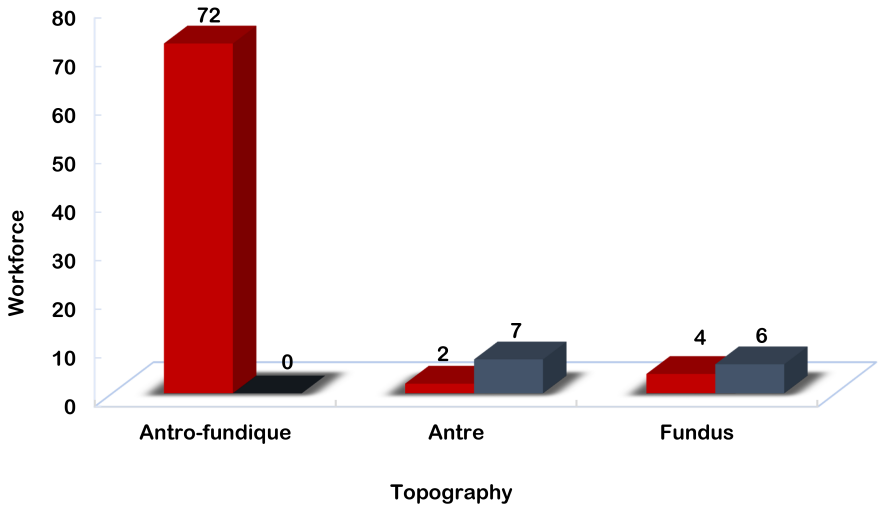

Figure 2 Tropism of *Helicobacter pylori* strains in the gastric mucosa. (■), Cag A+; (■), Cag A−.

Table 5 Correlation between strains and endoscopic lesions

	Stuff		
	Cag A+	Cag A–	
Variables	*n* (%)	*n* (%)	*P*-value
Endoscopic aspects			
Normal mucosa	53 (67.1)	12 (92.3)	0.08
Ulcerated lesion	14 (53.8)	—	0.2
Budding lesion	3 (11.5)	—	0.7
Frosted lesion	1 (3.8)	—	0.8
Erosive aspect	11 (42.3)	1 (7.7)	0.2
Congestive aspect	14 (53.8)	—	0.2
Infiltrated appearance	2 (7.7)	—	0.7
Acanthosis lesion	2 (7.7)	—	0.7
Other	3 (11.5)	—	0.7

Table 6 Correlation between strains and histopathological lesions

	Stuff		
	Cag A+ ($n = 78$)	Cag A– ($n = 13$)	
Chronic gastritis	*n* (%)	*n* (%)	*P*-value
Inflammatory activity	14 (17.90)	1 (9.10)	0.4
Glandular atrophy			
Slight	46 (58.97)	9 (69.23)	0.6
Moderate	15 (19.23)	3 (23.07)	
Severe	17 (21.80)	1 (7.70)	
Intestinal metaplasia	14 (17.9)	1 (9.1)	0.4
Dysplasia			
Low grade	—	1 (7.70)	0.3
High grade	2 (2.2)	—	
ADK	3 (3.3)	—	0.3
MALT lymphoma	—	—	

ADK, adenocarcinoma; MALT, mucosa-assisted lymphoid tissue.

Morocco and Bouh *et al*. in Mauritania reported a predominance in males.[15,16]

All age groups were affected, but the frequency was higher in younger patients, particularly those under 50, with a peak between the ages of 40 and 49 (32.97%). No significant correlation was found between age and Hp infection ($P = 0.567$). This result is similar to those reported in certain studies carried out in Africa, which found no significant correlation between the presence of Hp and age.[4,17]

Of the 43.86% of Hp-positive patients with abnormal endoscopy, there was a statistically significant difference between patients with infiltrating ($P = 0.0003$) and budding ($P = 0.01$) lesions. Carriage of Hp infection was exclusive in acanthosic (100%) and frosted (100%) lesions. In addition, Hp was strongly implicated in the occurrence of gastric ulcers, accounting for more than 80% of ulcers.

Carriage of the infection was 100% in severe GCA, in GC with inflammatory activity, and in GCA with intestinal metaplasia, and 75% of adenocarcinomas correlated with the presence of Hp. Our results are similar to those of Benoit *et al*. in France and Tagzout *et al*. in Algeria.[18-20]

Molecular analysis enabled us to determine the circulating strains on the basis of Cag gene expression. Of the 91 Hp-positive patients, 78 (85.71%) expressed the Cag A gene. This means a high prevalence of Cag A strains in the population. Kidd *et al*. in South Africa and Maeda *et al*. in Japan noted the presence of Cag A in all strains in their respective countries.[21,22]

The bacteria were found simultaneously in the antrum and fundus in 72 patients but isolated in the antrum and fundus in 9 and 10 patients, respectively. These results show that Hp does not have any preferential sites in the gastric tract, thus justifying systematic sampling of both sites. Our results are contrary to those of Attaf *et al*.[15] in Morocco[23] and Doh *et al*.[23] in Senegal, who report that Hp has a preferential site in the antral region.

Strains expressing the Cag gene were concurrently found in both sampling sites, whereas CagA-negative strains were found only in one site at a time (Fig. 2). This can be explained by the fact that bacteria carrying the Cag A gene have greater invasive and migratory properties.

In addition, 13 patients with normal mucosa at endoscopy had moderate to severe GCA with a focus of intestinal metaplasia. In addition, ulcerative and infiltrative lesions were the macroscopic appearance of gastric adenocarcinomas. There was a significant difference between normal mucosa and histopathological lesions (Table 5).

Cag A strains are present in considerably high proportions in moderate and severe GCA, in active GC, and in GC with foci of intestinal metaplasia. All adenocarcinomas are closely associated with the presence of Cag A strains (Table 6). These results prove the involvement of the Cag A protein in the occurrence of GCA and in the process of carcinogenesis of the gastric mucosa, as described in the literature.

A limitation of our study was the small size of our study population due to the COVID 19 pandemic. A large-scale study would have given us a better idea of the carriage and frequency of circulating strains.

In conclusion, Hp infection has a frequency of occurrence of 91% in the Congolese population, with a predominance in women. Clinical manifestations are polymorphous, dominated by epigastralgia, and the bacterium is mainly responsible for ulcers and gastritis. The histopathological lesions encountered in cases of Hp infection are chronic atrophic gastritis (active or inactive, metaplastic or not), and adenocarcinomas. Hp strains expressing the Cag A gene are found with high frequency in the Congo Basin. However, these strains do not have preferential sites in the gastric mucosa and are involved in the occurrence of severe endoscopic and histopathological lesions and in the process of carcinogenesis. The Cag A protein is highly pathogenic, making the strains circulating in Brazzaville highly virulent. Knowledge of circulating strains and their high pathogenicity will therefore be useful in improving the management of patients infected with Hp.

References

1 De Korwin JD. Epidemiology of *Helicobacter pylori* infection and gastric cancer. *Rev. Prat.* 2014; **64**: 189–93.
2 Hunt RH, Xiao SD, Megraud F. *Helicobacter pylori* in developing countries. World Gastroenterology Organisation Global Guideline. *J. Gastrointestin. Liver Dis.* 2011; **20**: 299–304.
3 World Health Organisation. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 61. Geneva: World Health Organisation. 1994; 177–240.
4 Malgalhaes DM, Luzza F. Epidemiology of *Helicobacter pylori* infection. *Helicobacter*. 2006; **11**: 1–5.
5 Suerbaum S, Michetti P. *Helicobacter pylori* infection. *N. Engl. J. Med.* 2002; **347**: 1175–86.
6 De Korwin JD, Lozzniewski A. *Helicobacter pylori*: basic concepts and perspectives. *EMC Gastroenterol.* 2000; **9**: 1–16.
7 Aguemon BD, Struelens MJ, Massougbodji A, Ouendo EM. Prevalence and risk-factors for *Helicobacter pylori* infection in urban and rural Beninese populations. *Clin. Microbiol. Infect.* 2005; **11**: 611–7.
8 Ankouane Andoulo F, Noah Noah D, Tagni-Sartre M, Ndjitoyap Ndam EC, Ngu BK. Epidemiology of *Helicobacter pylori* infection in Yaoundé: from peculiarity to African enigma. *Pan Afr. Med. J.* 2013; **16**: 115–20.
9 Werme K, Bisseye C, Ouedraogo I *et al*. Molecular diagnosis of *Helicobacter pylori* by PCR in patients undergoing gastroenterological consultation at the Saint Camille Medical Centre in Ouagadougou. *Pan Afr. Med. J.* 2015; **21**: 123–8.
10 Guantai M'itonga L, Nyerere Kimanga A, Wangari Ngugi C, Mutie TM. Association of *Helicobacter pylori* VacA gene polymorphisms and CagA gene with clinical outcome in dyspeptic patients in Kenya. *Int. J. Med. Health Sci.* 2015; **5**: 436–44.
11 Ontsira Ngoyi EN, Atipo Ibara BI, Moyen R *et al*. Molecular detection of *Helicobacter pylori* and its antimicrobial resistance in Brazzaville, Congo. *Helicobacter*. 2015; **20**: 316–20.
12 Itoua-Ngaporo NA, Mimiesse Monamou JF, Ahoui Apendi C *et al*. Epidemiological, clinical and endoscopic profil of *Helicobacter pylori* in two digestive endoscopy centers in Brazzaville. *Gastroenterol. Hepatol. Dig. Dis.* 2018; **1**: 1–5.
13 Ontsira Ngoyi EN, Guilloteau C, Benejat L *et al*. Cag A and Vac A *Helicobacter pylori* pathogenicity factors in Brazzaville, Congo. *Open J. Med. Microbiol.* 2019; **9**: 186–200.
14 Bommelaer G, Des varannes SB, Fléjou JF *et al*. CagA status and virulence of *Helicobacter pylori* strains. Results of a French multicentric prospective study. *Gastroenterol. Clin. Biol.* 2001; **25**: 1084–9.
15 Attaf N, Cherkaoui N, Choulli MK, Ghazali A, Mokhtari A, Soulaymani A. Epidemiological profile of *Helicobacter pylori* infection in the Gharb-Chrarda-Beni Hssen region. *Biol. Health*. 2004; **4**: 25–34.
16 Bouh KO, Jidou MT, El Bara A. Seroprevalence of *Helicobacter pylori* infection in an asymptomatic Mauritanian population. *Afr. J. Hepato-Gastroenterol.* 2015; **9**: 18–21.
17 Ilboudo D, Sangare L, Sanou J, Bougouma A, Diomande I. Epidemiological and clinical aspects of *Helicobacter pylori* infection in tropical areas. *Méd. Afr. Noire*. 1997; **44**: 24–8.
18 Bagny A, Darret T, Bouglouga O *et al*. *Helicobacter pylori* chronic gastritis at CHU Campus de Lomé. *J. Rech. Sci. Univ. Lomé*. 2014; **16**: 495–502.
19 Benoit A, Hoyeau N, Fléjou JF. Diagnosis of *Helicobacter pylori* infection on gastric biopsies: standard staining, special staining or immunohistochemistry? *Ann. Pathol.* 2018; **38**: 363–9.
20 Tagzout D, Tebaibia A. Histological diagnosis of *Helicobacter pylori* infection: prevalence and histopathological aspects. *Rev. Med. Interne*. 2019; **40**: A136.
21 Kidd M, Louw JA, Marks IN. *Helicobacter pylori* in Africa: observations on an 'enigma within an enigma'. *J. Gastroenterol. Hepatol.* 1999; **14**: 851–8.
22 Maeda S, Yoshida H, Ikenoue T *et al.* Structure of cag pathogenicity Island in Japanese *Helicobacter pylori* isolates. *Gut*. 1999; **44**: 336–41.
23 Doh K, Thiam L, Halim A, Takin RCA, Woto-Gaye G. Histopathological panorama of chronic gastritis with normal endoscopy in Senegal. *Trop. Med. Health*. 2017; **27**: 439–42.