

# HELICOBACTER PYLORI AND UPPER DIGESTIVE DISEASES - DIAGNOSIS THROUGH REAL TIME PCR

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## ABSTRACT

**Objective:** *Helicobacter pylori*, which has been designated by the WHO as type I carcinogen, has a global prevalence of over 50%. The aim of this study was to determine the association between this bacterium and upper gastrointestinal problems in an endoscopy unit in Ouagadougou, using a molecular diagnostic method.

**Methods:** This was a cross-sectional study of 111 patients who underwent upper gastrointestinal diagnostic endoscopy between April and June 2013. Each patient had four gastric biopsies taken. *Helicobacter pylori* was detected by real-time PCR at the Biomolecular Research Centre, CERBA/LABIOGENE.

**Results:** The prevalence of *Helicobacter pylori* in patients with various upper gastrointestinal problems was 84.7%. The use of medication that can reduce the *H. pylori* density was common among the infected patients, as history of antibiotics use, acid suppressant use and medications for eradication treatment were obtained in 43.6%, 26.6% and 18% respectively. Abdominal pain was the indication for the examination in 97.88%. The prevalence rate of *H. Pylori* was 87.21% among those who presented with signs of gastroesophageal reflux diseases (GERD) and 76% in those without signs. The endoscopy was normal in 55.85%. In all cases of ulcers (8.51%), the PCR was positive for *H. pylori*. There were no cases of gastric or duodenal cancers.

**Conclusion:** This study confirms the high prevalence of *Helicobacter pylori* infection in our cohort of patients when PCR method was used, but showed a direct relationship between presence of symptoms of gastroesophageal reflux disease and prevalence of *Helicobacter pylori* infection.

**Keywords:** *Helicobacter pylori*, Real-time PCR, Endoscopy, Prevalence.

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## INTRODUCTION

*Helicobacter pylori* is a bacterium with a helical shape (hence the name "*Helicobacter*") discovered in an area of the stomach near the pylorus ("*pylori*"). Since its culture by Barry Marshall and Robin Warren in 1982, *H. pylori* caused an upheaval of conceptions, not only

in gastroenterology but also in microbiology<sup>1</sup>. Since 1994, *H. pylori* is identified as type I carcinogen<sup>2</sup>.

The global prevalence of *H. pylori* is more than 50%<sup>3,4</sup>. In Burkina Faso, in the symptomatic patients who were seen in endoscopy, the infection rate by histology in adults was 82.6% in 1996<sup>5</sup> and

81.3% in 1997<sup>6</sup>. Among the asymptomatic people in Burkina Faso, the prevalence was 96.1% by serology in 2004<sup>7</sup>.

Infection occurs mainly in childhood<sup>4,7</sup>. In developing countries, most children are *H. Pylori* positive by the age of eleven. In developed countries, prevalence increases steadily with age due to a cohort effect (high in the elderly) and it is present in 20% to 90% of adult individuals according to age groups. The infection rate is lower in developed countries (30% of seropositivity). In developing countries, it reaches 80% to 90% in the general population.<sup>4,7-10</sup>

The mode of transmission is mainly inter-human through faecal-oral route and/or gastro-oral route (through saliva or vomit). A case of iatrogenic contamination was reported due to improper disinfection of medical equipment (endoscope, dental hygiene instruments)<sup>2,4</sup>. Genetic factors such as blood group O increases by 1.3 to 2 times the Relative Risk to a duodenal ulcer compared with other blood groups. Attachment of *H. pylori* to gastric epithelial cells strongly depends on the expression of Ag Lewis b which is itself dependent on the O status<sup>2</sup>.

*Helicobacter pylorus* is the subject of a sustained interest: genetic, epidemiological and pathophysiological progress has provided a better understanding of the infection. The latest international recommendations were published in October 2015<sup>11</sup>. In Burkina Faso the most *diagnostic* methods used were the urease test and histology; clinical manifestations of the bacterium are still poorly known<sup>5-7</sup>.

The Polymerase Chain Reaction (PCR) is a molecular technique for the diagnosis, but it is limited due to its inaccessibility and high cost. However, several types of PCR (standard, real-time, multiplex) and several kits for the diagnosis of this bacterium are available on the market. Only one study in 2012 used classic PCR for the diagnosis of the pathogenic microorganism in Burkina Faso<sup>12</sup>. The Real time PCR (RT PCR) technology has become more famous and it is used in the detection and quantification of fluorescent "reporter" proportionally of the "amplicons" quantity generated during PCR. Its permit makes it possible to quickly obtain specific and quantitative results by using complete automatic procedure<sup>13</sup>

The aim of this study was to investigate the association of upper gastrointestinal problems with

the presence of the bacterium using real-time PCR (Taqman assay).

## **MATERIALS AND METHODS**

Our cross-sectional study was conducted in various settings which included the following: the Endoscopy Unit of the Medical Centre located in the General Sangoulé Lamizana camp (CMGASL), the laboratory of Saint Camille Medical Centre (CMSC) and the Pietro Annigoni Biomolecular Research Centre (CERBA/LABIOGENE) for the *H. pylori* molecular analyses.

Patients who consented to participate in the study, and went through a diagnostic upper gastrointestinal tract (GIT) endoscopy between April 22 and June 27, 2013 in the relevant centres were included.

All patients were interviewed to collect socio-demographic data (age, sex, residence, occupation, socioeconomic status) and clinical data (history, risk factors and symptoms). Occupation was *considered* as a risk factor for the infection.

Patients' means of regular transport was used as the basis for their socio-economic status estimation. Those who used cars were considered as having a high socioeconomic status; those who use motorcycles were considered as having medium socioeconomic status and finally those who use bicycles or go by foot as being of low socioeconomic status.

Family size and the number of "siblings" sleeping in the same bed or in the same room during childhood were risk factors. Cousins were included in the term "siblings".

The usual place where patients took their meals was recorded. Based on place of meal consumption, the patients were classified into 2 (Those who took their meals outside the home including those taken in makeshift restaurants, uncontrolled or open places (road sides) and those who took their meals at home or in restaurants inspected or approved by the sanitary agencies). This classification was used as surrogate for the level of hygiene.

The GIT symptoms sought were abdominal pain (type and location), clinical signs of Gastroesophageal Reflux Disease (GERD): heartburn, acid regurgitation, belching, clinical signs of dyspepsia: bloating, nausea, vomiting, halitosis, feeling of slow digestion or gastric fullness.

Tissue specimens were collected during upper GIT endoscopy; four biopsies were taken by one of the two gastroenterologists of the unit: two biopsies were taken from the antrum and two from the gastric fundus.

The brand of the endoscope used for the study was Olympus® Evis Exera GIF-Q180.

Premedication was with oral Xylocaine gel as local anesthetic (gargled by the patient). All data were collected on a predetermined sheet. The samples were placed in sterile Eppendorf tubes and then frozen at -20 °C in the Saint Camille Medical Centre laboratory.

### **DNA Extraction and *H. pylori* Detection by Real-Time PCR**

DNA extraction from gastric biopsies was done using the *DNA-SorbC*® kit (Sacace Biotechnology, Como, Italy) following the protocol supplied by the manufacturer. The resulting DNA was quantified using a nanodrop (Biodrop®).

The molecular diagnosis of *H. pylori* was performed by real-time PCR using the *H. pylori* Real Time TM® kit (Sacace Biotechnologies Como, Italy) in a Cyclo-96® Real Time PCR machine (Sacace Biotechnologies).

Real-time PCR method used was a Taqman assay. The protocol provided by the manufacturer has been complied with: preparing the quantity of reaction tubes required for the samples and controls (+2). In a sterile PCR tube and for each sample, the reaction volume was: 10µl of PCR-mix-1FRT ; 5.0µl of PCR-Buffer-FRT ; 0.5µl of TaqF DNA Polymerase and 10µl of DNA sample previously extracted or 10µl of DNA-buffer for the negative control, 10µl of *H. pylori* DNA for the positive control, 10µl of internal control for the positive amplification control.

PCR condition: one cycle at 95 °C/15 min and 45 cycles at 95 °C/10s, 60 °C/30s, 72°C/10s. *H. pylori* was detected on the JOE channels (Yellow)/Hex/Cy3, internal control of the DNA on the FAM/Green channels. The value of thresholds in each channel was 40.

Data were entered into Epi Info and analysed using SPSS 17.0 and Epi Info 3.5.1 software. Chi square test was used for comparison. The difference was significant at  $p < 0.05$ .

We received approval from the Ethics Committee of the Saint Camille Medical Centre and CERBA/LABIOGENE before carrying out the study. Each person involved in this study gave informed consent.

## **RESULTS**

### ***Social and demographic Data***

During the study period 114 diagnostic gastrointestinal endoscopies were performed in the selected centers for the study. Three patients who did not give consent were excluded. Therefore, 111 patients (97.37%) were included in the study population. All biopsies were performed in all patients. There were no complications. Table 1 summarizes the social and demographic characteristics of the study population.

#### *Prevalence of the infection*

PCR was positive in 94/111 patients or a prevalence rate of 84.7%. The average age of patients infected with *H. pylori* was 37.42 years  $\pm$  12.13. The youngest was 17 and the oldest was 71. with *H. pylori* was 37.42 years  $\pm$  12.13. The youngest was 17 and the oldest was 71.

#### *H. pylori carriage study*

Tables 2 to 4 summarize the relationship between *H. pylori* carriage and certain factors associated with the infection.

**Table 1: Presence of *H. pylori* depending on risk factors**

Risk Factors	Presence of <i>H. pylori</i>		P
	Negative <i>H. pylori</i> (%)	Positive <i>H. pylori</i> (%)	
<b>Age group (Years)</b>			
< 20	0 (0)	4 (100)	0.34
20 – 40	15 (19.2)	63 (80.8)	
40 – 60	2 (7.7)	24 (92.3)	
> 60	0 (0)	3 (100)	
<b>Gender</b>			
Male	5 (10.2)	44 (89.8)	0.1
Female	12 (19.4)	50 (80.6)	
<b>Origin</b>			
Burkinabes	16 (15.1)	90 (84.9)	0.79
Other Africans	1 (25)	3 (75)	
European	0 (0)	1 (100)	
<b>Place of residence</b>			
Urban	9 (12.16)	65 (87.84)	0.1
Rural	8 (21.62)	29 (78.38)	
<b>Occupation</b>			
Artisan	0 (0)	1 (100)	0.13
Farmer	0 (0)	8 (100)	
Pupil-Student	1 (7.7)	12 (92.3)	
Informal Sector	3 (8.83)	31 (91.17)	
Middle Manager	1 (10)	9 (90)	
Military-paramilitary	3 (17.65)	14 (82.35)	
Housewife.	8 (32)	17 (68)	
Senior Manager	1 (33.34)	2 (66.66)	
<b>Socio-economic status</b>			
Low	6 (15.38)	33 (84.62)	0.1
Medium	10 (15.38)	55 (84.62)	
High	1 (14.28)	6 (85.72)	
<b>Alcohol Consumption</b>			
Yes	4 (14.81)	23 (85.19)	0.48
No	13 (15.48)	71 (84.52)	
<b>Tobacco Consumption</b>			
Yes	0 (0)	10 (100)	0.09
No	17 (16.83)	84 (83.17)	

**Table 2:** Presence of *H. pylori* depending on risk factors

Risk Factors	Presence of <i>H. pylori</i>		P
	Negative <i>H. pylori</i> (%)	Positive <i>H. pylori</i> (%)	
<b>Share same bed</b>			
≤ 2	7 (13.72)	44 (86.27)	0.15
3-5	6 (12.76)	41 (87.23)	
>5	2 (25)	6 (75)	
No answer	2 (40)	3 (60)	
<b>Share the same room</b>			
≤ 2	5 (16.13)	26 (83.87)	0.27
3 to 5	6 (10.17)	53 (89.83)	
> 5	3 (20)	12 (80)	
No answer	3 (50)	3 (50)	
<b>Number of siblings</b>			
0	1 (100)	0 (0)	0.01
1 to 6	11 (16.92)	54 (83.07)	
7 to 11	2 (6.25)	30 (93.75)	
> 11	3 (23.08)	10 (76.92)	
<b>Water from wells</b>			
Yes	0 (0)	8 (100)	0.12
No	17 (16.50)	86 (83.50)	
<b>Water from ponds</b>			
Yes	0 (0)	4 (100)	0.25
No	17 (15.89)	90 (84.11)	
<b>Tap water</b>			
Yes	12 (15.19)	67 (84.81)	0.47
No	5 (15.62)	27 (84.37)	
<b>Borehole water</b>			
Yes	6 (19.35)	25 (80.64)	0.23
No	11 (13.75)	69 (86.25)	
<b>Filtered Water</b>			
Yes	1 (33.33)	2 (66.66)	0.23
No	16 (14.81)	92 (85.18)	
<b>Blood Group O</b>			
Yes	0 (0)	10 (100)	0.09
No	17 (14.81)	84 (85.18)	
<b>Place of meals</b>			
In family	13 (18.84)	56 (81.16)	0.35
Outside	3 (13.64)	19 (86.36)	
Equal frequency	0 (0)	14 (100)	
Not specified	1 (16.66)	5 (83.33)	

**Table 3:** Effect of drugs on prevalence of *Helicobacter pylori*

Drug	Presence of <i>H. pylori</i>		P
	Negative <i>H. pylori</i> (%)	Positive <i>H. pylori</i> (%)	
<b>Ingestion of NSAIDs</b>			
Yes	8 (17.02)	39 (82.98)	0.34
No	9 (14.06)	55 (85.94)	
<b>Antibiotic treatment</b>			
Yes	5 (10.87)	41(89.13)	0.97
No	12 (18.46)	53 (81.53)	
<b>Antisecretory treatment</b>			
Yes	2 (7.4)15	25 (92.6)	0.88
No	13 (17.85)	69 (82.15)	
<b>Anti-<i>H. pylori</i> Treatment</b>			
Yes	2 (10.53)	17(89.47)	0.97
No	15 (16.3)	77 (83.7)	

**Table 4:** Relationship between upper gastrointestinal symptoms and *Helicobacter pylori* infection

Symptom	Presence of <i>H. pylori</i>		P
	Negative <i>H. pylori</i> (%)	Positive <i>H. pylori</i> (%)	
<b>Abdominal Pain</b>			
Yes	16 (14.81)	92 (85.18)	0.23
No	1 (33.33)	2 (66.66)	
<b>GERD Symptoms</b>			
Yes	11 (12.79)	75 (87.21)	0.1
No	6 (24)	19 (76)	
<b>Nausea</b>			
Yes	6 (12.76)	41 (87.23)	0.27
No	11 (17.19)	53 (82.81)	
<b>Halitosis</b>			
Yes	8 (17.39)	38 (82.61)	0.31
No	9 (13.85)	56 (86.15)	
<b>Slow digestion feeling</b>			
Yes	5 (20)	20 (80)	0.23
No	12 (13.95)	74 (86.05)	
<b>gastric fullness feeling</b>			
Yes	0 (0)	10 (100)	0.08
No	17 (16.83)	84 (83.17)	
<b>Bloating feeling</b>			
Yes	0 (0)	4 (100)	0.66
No	17 (15.89)	90 (84.11)	

The average age of patients was 37.42 years and 63 infected patients were between 21 and 40 years old. Prevalence was 100% in patients aged under 21 and those over 60 years.

The sex ratio was 0.88 in infected patients. Prevalence was substantially equal regardless of socioeconomic status. Ten (10) people who were active smokers were all infected ( $p=0.09$ ).

Antibiotic medication taken within one month preceding the examination was reported by 43.6% of patients infected, with a prevalence of 89.13%. The name of the prescribed antibiotic was not found in 60.97%. Amoxicillin and metronidazole were found in the other cases in order of decreasing frequency (58.82% and 41.18%). Use of acid suppressant within two weeks prior to examination was reported by 26.6% of infected patients and the infection rate among them was 92.6%. Treatment to eradicate *H. pylori* within the two weeks prior to the examination was reported by 18% of patients infected, with a prevalence of 89.47%.

One female patient had no brother or sister (only daughter) and the PCR was negative for her. The link between the number of "siblings" and the infection was significant with  $p=0.01$ .

Sometimes, the drinking water sources were diversified for the same patient. All patients who consumed well water or backwater were infected. All patients with blood group O were infected ( $p = 0.09$ ).

Prevalence was 81.16% in patients who usually took their meals at home; 86.36% in those took their meals outside and 100% in case of equally between these different places.

Abdominal pain was present in 97.88% of our patients. Epigastric burning sensation occurring during fasting was the predominant indication for gastroscopy.

At least a symptom of GERD (heartburn, regurgitation) was found in 79.78% or 4/5<sup>th</sup> of the infected patients. Patients frequently reported several symptoms at once. The prevalence rate was 87.21% among those who had signs of GERD and 76% among those who didn't have such signs ( $p=0.1$ ).

Several symptoms of dyspepsia were often reported by the same patient. Nausea and halitosis were the most common. All patients with bloating (04) or a feeling of tension or epigastric fullness (10) were all infected.

### Endoscopic Results

Approximately 84% *H. pylori* infected patients had normal upper gastrointestinal endoscopy while 85.71% had endoscopic abnormality ( $p=0.4$ ).

Several anomalies were sometimes found in the same patient. The most common were hiatal hernia found in twenty (21.28%) infected patients and reflux esophagitis in sixteen (17.02%) infected patients. Erosions or mucosal erythema were found in ten (10.63%) patients. The eight patients (8.51%) who had ulcer were infected with *H. pylori*: six (6.38%) bulbar and two (2.13%) gastric. There was no case of oesophageal, gastric or duodenal cancer. There were two (2.13%) cases of polyps, looking benign in appearance and one case of micronodules (1.06%).

### DISCUSSION

The centre likely has selection bias due to its location in a military camp. Military and paramilitary however constituted only 15.3% of our sample.

Our results showed a high frequency of *H. pylori* infection in patients with digestive signs (84.7%). The prevalence rates found in Burkina Faso over the past twenty years vary between 78% and 96.1%<sup>5-7,12,14</sup>. The prevalence rates of some African studies were between 62% and 89.4%<sup>15-18</sup> which are clearly greater than the European data between 5% and 50%<sup>7,10,19,20</sup>. Real time PCR was used in this study to improve the diagnostic efficacy<sup>13</sup>.

In our study, the sex ratio was 0.88 in infected patients. Prevalence trends are mixed in scientific papers published: male predominance<sup>18</sup> or female predominance<sup>16</sup>, and no gender difference<sup>2,5,6,17,21</sup>.

Occupation and socioeconomic status were not significantly linked to the infection in our patients. Ilboudo et al reported in 1997 that "in Africa, every individual taken at their adult age has spent their childhood in an environment conducive to contamination"<sup>6</sup>. Several studies suggest that contamination occurs early in childhood in developing countries and that at the age of eleven, the majority of children are infected<sup>2,4,8-10,21</sup>. Some social and cultural factors have been implicated in this contamination like mothers pre-chewing food before giving them to their children<sup>2,10,14,21</sup>.

In our study, infection was significantly related to the size of the family and specifically to the number

of "siblings" with  $p=0.01$ . We have not assessed the impact of the common plate or glass. However, prevalence was higher in crowded households where hygiene was questionable. Consumption of water from wells or ponds or meals taken in makeshift restaurants, uncontrolled or open places (by the roads). Congestion/proximity and lack of hygiene are known risk factors for the infection<sup>2,7,10,20,21</sup>.

In our study, there was no significant link between alcohol or tobacco and infection. This finding was already noted by Brown in 2000 in an epidemiological review<sup>22</sup>. According to literature, NSAIDs ingestion is an aggravating factor for ulcer and ulcer bleeding in those with the infection<sup>2,19,23-25</sup>.

The bacterial population density is heterogeneous and can be affected by previous treatment with antibiotics or proton-pump inhibitors<sup>8,24,25</sup>. In our patients, these situations were common: 43.6% for the antibiotic treatment, 26.6% for anti-secretory treatment and 18% for eradication treatment, but these did not significantly affect the prevalence of infection. The explanation is possibly due to the high sensitivity of PCR in diagnosing *H. pylori* infection. For the diagnosis of the bacterium, the specificity of the PCR compared to other methods is established<sup>18,13,24-27</sup>. Weiss J *et al*<sup>28</sup> showed that PCR detected *H. pylori* with 94% of sensitivity and 100% of specificity. Besides, a study conducted in 2012 in Burkina Faso<sup>12</sup> found on the same samples the following prevalence rates: 88.57% by Immunocomb serology and 91.43% by classic PCR. In 2005, Ching-Chu *et al*<sup>23</sup> showed the contribution of PCR in bleeding ulcer cases. This allows us to assume there would have been several false negative if the diagnosis was not made using one of the other commonly used methods such as histology. The molecular technique would not be necessary in some cases to improve diagnostic efficiency, despite its high cost. Given the small size of the series in symptomatic patients, epidemiological studies are needed to provide answer to this question.

Pain was the most common indication for upper gastrointestinal endoscopy. It was present in almost all patients (97.88%); it was commonly an epigastric burning sensation occurring when hungry. Ilboudo *et al*<sup>6</sup> had made the same observation in symptomatic patients. Still in Burkina Faso, Cataldo *et al*<sup>7</sup> had found a 96.1% prevalence rate in asymptomatic patients. The infection is essentially asymptomatic<sup>2,8,19,24</sup>.

Pain is nonspecific and must lead to searching for organic disorders.

Infected patients frequently had clinical manifestations of GERD (frequency = 4/5 and prevalence = 87.21%). Epidemiological studies in Europe showed an inverse relationship between the prevalence of *H. pylori* infection and GERD although the protective effect of the infection was not demonstrated<sup>24,25</sup>. The high frequency of GERD manifestations was probably linked to that of hiatal hernia (75.26 %), but this direct relationship requires further study.

In our study, all patients who reported bloating or a feeling of tension or epigastric fullness were infected. Infected patients often reported nausea and halitosis. *H. Pylori* is now a new cause of dyspepsia<sup>8</sup>.

The normal endoscopic aspect despite the presence of the bacterium is known and is explained by asymptomatic chronic gastritis. This aroused the interest in a systematic research of the bacterium, or systematic eradication in case of upper gastrointestinal symptoms in countries with high prevalence of *H. pylori*<sup>8</sup>. Additional studies (virulence factors, *H. pylori* genotypes, cofactors) could provide some answers. Pending the findings of these studies, the last recommendations<sup>8,11</sup> remain the reference.

The frequency of peptic ulcer in our patients was 8.51%; which is significantly lower than the rate of 21.7% found by Ilboudo *et al*<sup>5</sup> 20 years ago. *H. pylori* was found in all our ulcer cases. Ilboudo and *et al*<sup>6</sup> had suggested a systematic eradication in cases of peptic ulcer.

There was no case of cancer. It is still an enigma why few strains by certain genes (*jhp0940*, *jhp0945*, *jhp0947*, *jhp0949*) in *H. pylori* low prevalence region are associated with severe diseases<sup>29,30</sup>. Our data supported this enigma, which is still incompletely understood. Studies on the possible protective role of some environmental and dietary factors, or the study on circulating bacterial strains are still awaited. Histological analysis must be combined to clarify the impacts of these factors.

## CONCLUSION

*Helicobacter pylori* prevalence is very high in Burkinabe patients who have upper gastrointestinal symptoms. Contrary to expectation, a direct relationship between symptoms of GERD and prevalence of *H.*

*pylori* was demonstrated in this study. More studies are warranted to further elucidate this interesting observation.

This work has also raised the need for bacteriological studies focusing on sensitivity to antibiotics; identification of virulence strains and factors; or protection (food, environmental) against circulating *H. pylori* in Burkina Faso.

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