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Research Article

Biochemical and resistance profile of Helicobacter pylori isolated in N'Djamena in Chad

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Abstract

Helicobacter pylori infection and resistance to antibiotics is a public health problem. The objective of this study was to determine the prevalence and rates of resistance to antibiotics used in the protocol for the management of patients with *Helicobacter pylori* infection.

Spanning a period from February 2020 to February 2021, it was an observational diagnostic study on gastric biopsies and stool including 97 patients admitted for endoscopy. It was carried out according to standard methods of medical microbiology.

Of 97 patients whose mean age was 46.10 years with extremes of 16 and 85 years, an infection prevalence of 60.82% was observed. 44.07% of infected patients were between 16 and 39 years old, 33.90% between 40 and 63 years old, and 22.03% between 63 and 85 years old.

Significant differences were observed between the proportions of positive (81.44%) and negative (18.56%) cultures, between infection with *H. pylori* (75%) and other microbial agents (25.35%) with probabilities of 0.01 and 0.02 respectively.

The most resistant antibiotics were: Metronidazole, Clarithromycin, Levofloxacin, Tetracycline and Amoxicillin with resistance rates of 74.58%, 16.95%, 13.56%, 8.47%, and 5.08% respectively. The frequencies of antibiotic resistance revealed 74.58% for Metronidazole, 16.95% for Clarithromycin, 13.56% for Levofloxacin, 8.47% for Tetracycline, and 5.08% for Amoxicillin.

This study made it possible to determine a significant proportion of *H. pylori* infection and to shed light on the resistance to the antibiotics used in the eradicating treatment of the bacteria. From this study, we retain that the prescription of Metronidazole is prohibited in Chad.

Keywords: *Helicobacter pylori*, Resistance, Antibiotic, Chad.

INTRODUCTION

Helicobacter pylori is a helical, micro-aerophilic Gram-negative bacterium whose main reservoir is the human stomach [1]. Present in 20 to 30% of the population of industrialized countries, and in 70 to 90% of the inhabitants of developing countries, it was the most widespread bacterial infection in the world with a prevalence of 50% of the world population in 2016 [2-5]. It is the main agent responsible for chronic gastritis and peptic ulcers, and is also involved in the onset of stomach cancer (class 1 carcinogen according to the WHO in 1994) in 1 to 3% of infected cases (adenocarcinoma and MALT lymphoma) [6, 7].

Discovered in 1875 by German researchers, it was then highlighted and characterized for the first time in 1982 by two Australian researchers (Nobel Prize for Medicine in 2005: J. Robin Warren and Barry J. Marshall) [1]. Thirty years later, medicine is faced with the problem of H. pylori's resistance to antibiotics. This resistance is mainly to clarithromycin, a very effective macrolide when administered in combination, but also to imidazoles, fluoroquinolones and to a lesser extent other antibiotic [8, 9].

In fact, the objective of achieving eradication in all patients treated with the first therapeutic approach, a situation usual for common infectious diseases, has not been achieved for H. pylori. The initial sensitivity of *H. pylori* to both clarithromycin and imidazoles, essential drugs for first-line triple therapy, was gradually reduced. Likewise, the low rate of resistance of H. pylori to the fluoroquinolones primarily used for second-line therapy observed in the past has

increased over the past decade, while rates of resistance to against amoxicillin and tetracycline seem to have remained weak [10].

Antibiotic resistance has a crucial role in the management of H. pylori infection, it is the main cause of treatment failure. Therefore, knowing resistance can help develop new management strategies to improve treatment success, it can also help prevent resistance.

In Chad, data on the resistance of *H. pylori* to antibiotics is not yet available, but infection was frequent with a prevalence of 83.7% in dyspeptic patients undergoing upper gastrointestinal endoscopy at the Internal Medicine Service. and Gastroenterology of the General National Reference Hospital of N'Djamena in 2010. Hence the need to study resistance, which is the main cause of therapeutic failure [11]

The general objective of the present study is to contribute to improving the management of patients with Helicobacter pylori infection. To achieve this objective, we have set ourselves the following specific objectives:

- ✓ Determine the prevalence of Helicobacter pylori infection in symptomatic patients admitted to the HGE Department of the CHU-RN;
- ✓ Identify the strains of H. pylori and determine their resistance to the antibiotics used in the PEC protocol.

MATERIAL AND METHODS

Study framework

This work was carried out at the Hepato-Gastroenterology (HGE) department, at the Biomedical Analysis Laboratory of the National Reference University Hospital (CHURN) of N'Djamena, and at the Research, Diagnosis and Scientific Expertise Laboratory. (Labo-ReDES) of the Faculty of Human Health Sciences (FSSH).

Type and period of study

This was a prospective, observational, descriptive and analytical diagnostic study that lasted 12 months from February 2020 to February 2021.

Study population

Were included in this study all patients of all sex and age admitted to the Hepato-Gastroenterology department of the CHU-RN, presenting symptoms suggestive of an infection with *H. pylori*.

Inclusion criteria

- ✓ Indication and performance of digestive endoscopy;
- ✓ Verbal consent of the patient or his beneficiary.

Non-inclusion criteria

- ✓ The impossibility, contraindication or non-performance of digestive endoscopy or gastric biopsy samples;
- ✓ The patient's refusal (or his successor in title) to participate in the study;

In the event of treatment in progress or in the four weeks preceding endoscopy, with Proton Pump Inhibitors (PPIs), Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) or antibiotics.

Sampling

Sampling for convenience in relation to the duration of the study.

Study variables

- ✓ The status of patients on *H. pylori* infection;
- ✓ Gender, age, profession, origin of patients, notion of self-medication with antibiotics, reason for consultation, result of endoscopy;
- ✓ The sensitivities and resistance of the different strains to the different antibiotics mentioned.

Biopsy and stool collection

The gastric biopsy was done during the endoscopy performed by the hepato-gastroenterologist, and it was in the precondition of fasting at least 8 hours. All procedures were explained to patients or their beneficiaries. They were free to consent or refuse. Two biopsies (antral and fundal) were taken from each patient using gastrointestinal endoscopic biopsy forceps. The transport between the Hepato-Gastro-Enterology Service (SHGE) and the laboratories was done immediately after the sample. The biopsies were transported in 5 mL tubes containing 1.5 mL of saline. The whole was transported in a Cool box to the CHURN laboratory for analysis.

The stools were collected by the patients in sterile vials.

CULTURE

We used four types of culture media in this study. These were pylori agar supplied to us by BioMérieux, chocolate agar (GC), Columbia blood agar (GCS) and Mueller Hinton blood agar ((GMHS) that we prepared in the laboratory. For these two Last, we used the base agars (Columbia agar and Mueller Hinton agar), presented as lyophilized powder and fresh sheep blood as. Mueller Hinton blood agar was used for the antibiogram, the other three for the culture. of *Helicobacter pylori*.

We then added 10 mL of mixture of VCN (Vancomycin, Colistin, Nystatin) antibiotics and selective antifungals in the culture media to inhibit the growth of molds and unwanted bacteria.

Preparation and inoculation of samples

In the laboratory, in order to avoid contamination, most of the handling was carried out under a microbiological safety hood. Each biopsy was dilacerated with a scalpel in a petri dish to be inoculated in three media (G. pylori, GCS, and GC), one part used for Gram stain, and a last part for the urea test. The stools were also inoculated in different culture media.

From the dilaceration product, we deposited a suspension in the center of each culture medium (G. pylori, GCS, and GC) and we spread by several framing using the loop so that the entire half-surface of the box is seeded without touching the edges of the box (Photo: Table). Each culture medium was incubated at 37 ° C under a micro-aerobic atmosphere (Photo: Table). The culture was checked every 3 days from the 1st day until 12 days. A positive *H. pylori* culture was explained by the appearance of small round, domed and transparent colonies (Photos 9 and 10). It was confirmed by microscopic observation after Gram stain where *H. pylori* appear pink in spiral form, and by biochemical tests (catalase +, oxidase + and urease +++). A negative culture was confirmed 12 days after incubation when the criteria described for a positive culture were absent.

Detection of urease activity

Part of the debris from the biopsy or stool was suspended in 100 µL of urea medium and placed at 37 ° C for 24 hours. In

the presence of the bacteria, a colorimetric change from red from phenol to pink is observed.

GRAM staining

On a part of the product of laceration of the biopsy or of the stool, we had carried out a direct examination after staining of GRAM. For this, we deposited part of the said product on a slide and then spread in such a way as to obtain a smear, which, after drying, we carried out the GRAM staining.

On microscopic observation, at the objective x 100, we understand the morphology and the color of bacteria. Colonies of *H. pylori* appear curved, spiraling, and pink.

Study of the sensitivity of H. pylori isolated from diarrhea and biopsies to antibiotics

The antibiogram was carried out manually by the Kirby and Bauer method, which is the method by diffusing discs impregnated with antibiotics in agar medium, and measuring the diameter of inhibition (Photos: Table). In our study, the antibiotics we were interested in were in Table 1.

Inoculation procedure

Within 15 minutes of adjusting the turbidity of the inoculum suspension, a cotton swab was dipped into the suspension. The soaked swab was squeezed firmly by twisting it against the bottom wall of the tube just above the liquid level to remove excess inoculum. Then it was spread three (3) times over the entire surface of the agar, rotating the dish approximately 60 °, after each application, to obtain an even distribution of the inoculum. Finally, all over the edge of the agar surface was swabbed.

Procedure for dispensing antibiotic-impregnated discs

6.35 mm diameter blotting paper discs impregnated with a determined load of antibiotic were used for the antibiogram tests. 5-10 min after the inoculum, the antibiotic discs were applied to the Petri dishes. We place the discs individually with sterile forceps or using a dispenser against the agar. The number of discs per Petri dish must be such that the zones of inhibition do not intersect in order to allow reading of the diameters in several directions. The number of discs chosen varies between 6 to 7 per box of 90 mm. Once the discs have been placed on the agar, they are left at laboratory temperature (25 ° C) for about 30 minutes and then we bring them to incubation for 24 hours at 37 ° C. After overnight incubation, we measure the diameter of each zone of inhibition (including disc diameter) in mm using a graduated measuring instrument called a caliper.

Choice of antibiotics required for susceptibility testing of isolated *H. pylori*

The data applied for the reading comes from recognized methods of the Committee of Antibiogram of the French Society of Microbiology and of the National Committee on Clinical Laboratory Standards (CA-SFM, 2016-2020; NCCLS, 1998). Table 1 below gives us the list of antibiotics, their charges and the limits of the diameters.

Table 1: Interpretation guide for antibiotic inhibition diameters (CA-SFM 2016-2020; NCCLS, 1998).

Antibiotics	**Concentrations (mm)**	**Resistant (mm)**	**Intermediate (mm)**	**Sensible (mm)**
Clarithromycin **(CLA)**	2µg	Ø < 19	19-20	Ø ≥ 20
Amoxicillin (AML)	20µg	Ø < 19	19-23	Ø ≥ 23
Amoxicillin + Clavulanic acid (AMC)	20/10µg	Ø < 19	19-22	Ø ≥ 22
Lévofloxacin 5 µg (LEV)	5µg	Ø < 19	19-21	Ø ≥ 22
Tetracycline (TET)	30 UI	Ø < 17	17-19	Ø ≥ 19
Metronidazole (MET)	5µg	Ø < 11	11-13	Ø ≥ 13

Data processing

The collected data was entered and analyzed using Word 2013 and Excel 2013 software. Statistical analysis used the chi-square (χ^2) test to compare two qualitative variables. The p-value ≤ 0.05 was considered significant.

RESULTS

Overall prevalence of infection

A total of 97 patients were included in this study. These were 56 men (57.73%) and 41 women (42.27%) with a sex ratio of 1.4 an average age of 39.6 years with extremes of 16 and 85 years. After the culture on the biopsies, it resulted that out of the 97 patients sampled, 59 or 60.82% were positive for *H. pylori*, and 38 patients or 39.18% were negative.

Distribution of infection by gender

The distribution of infection by gender showed that the frequencies of positive cases by sex compared to the total of positive cases. We deduce that among the 59 positive cases, 57.63% (n = 34) were men and 42.37% (n = 25) women with a sex ratio of 1.36. Processing of these data provided us with a chi-square of 0.0095 below the significant critical threshold (3.84) of 5%. This allowed us to state that with a margin of error of 0.05, the occurrence of *H. pylori* infection is not related to sex.

Distribution of infection by age group

The figure below illustrates the distribution of the 59 infected patients by age group. The most represented age group was 16 to 39 years old with 26 cases or 44.07%. Processing of these data allowed us to determine a chi-square of 0.752 which was below the critical value (5.99) at the significant threshold of 5%. So, we could say that with a margin of error of 0.05, the occurrence of *H. pylori* infection is not related to age. The median age was 46.10 years with extremes of 16 and 85 years.

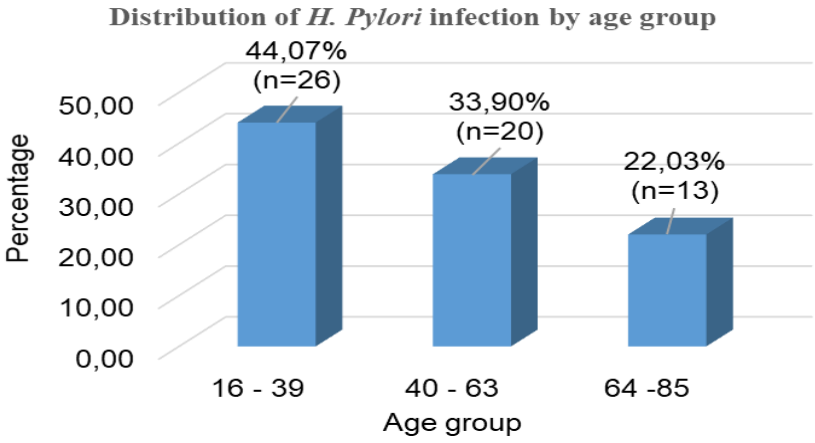


Figure 1: Distribution of *H. Pylori* infection by age group

Breakdown of infected patients by category

This figure illustrates the distribution of infected patients by category. Hospitalized patients represented 64.41% with a number of 38 patients. In fact, the patients undergoing endoscopy at the CHU-RN were mostly hospitalized people.

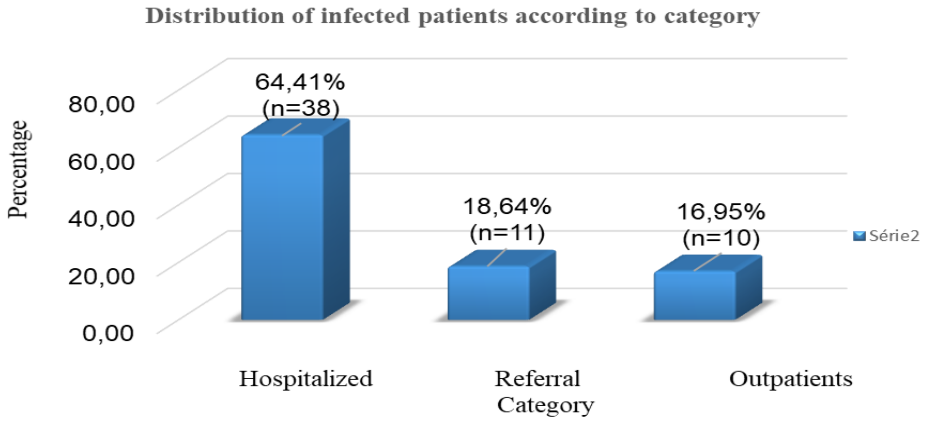


Figure 2: Distribution of positive cases by category

Distribution of infected patients by self-medication with antibiotics

Among the 59 infected patients, 33 or 55.93% said that they do not use antibiotics without a medical prescription, 14 or 23.73% said they were in the habit of using antibiotics, and 12 or 20, 34% had ignored.

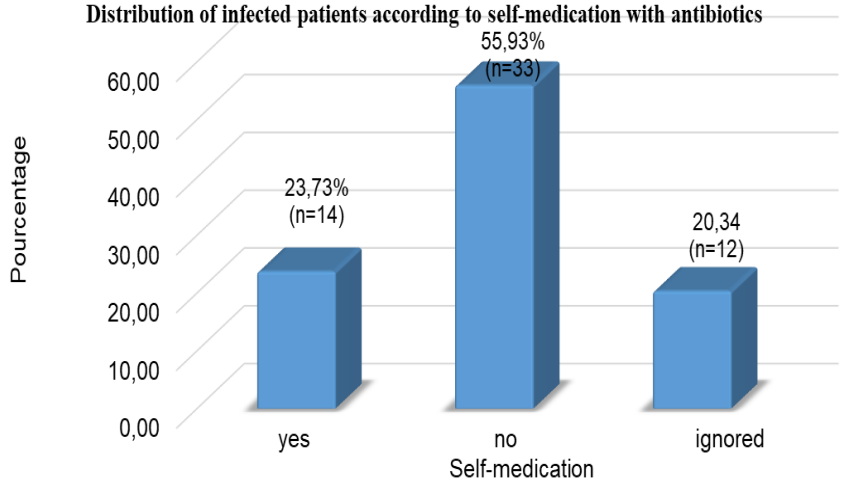


Figure 3: Distribution of positive cases according to self-medication

Breakdown of infected patients by reason for consultation

This table shows the distribution of positive patients according to the reason for consultation. In this study, it was found that 32 patients or 54.24% had consulted for epigastralgia.

Table 2: Distribution of infected patients according to the reason for consultation

Indication	n	%
Epigastralgia	**32**	
Pyrosis	9	15,25
Dyspepsia	4	6,78
Others*	14	23,73
Total	**59**	**100**

n = number; % = percentage; * (Nausea, vomiting, hematemesis, regurgitation)

Distribution of positive cases according to the endoscopy result

Of the 59 people infected, endoscopy revealed that 8.47% had normal mucosa. Among the lesions suggestive of *H. pylori* infection, we recorded 88.14% of gastritis, and high rates of gastric and duodenal ulcers with frequencies of 57.63% and 45.76% respectively, then 10.17% pseudo-nodular gastropathy.

The results are shown in the table below.

Table 3: Distribution of infected patients according to the endoscopy result.

Gastroscopy result	n	%
Gastritis	**52**	**88,14**
Peptic esophagitis	49	83,05
Gastric ulcer	34	57,63
Duodenal ulcer	27	45,76
Varicose veins	7	11,86
Pseudonodular gastropathy	6	10,17
Normal mucosa	5	8,47

n = number, % = percentage

Prevalence of bacteria isolated in different culture media

After culturing each biopsy in 3 different media, we obtained the result shown in Table 4 below. *Pseudomonas*, Streptococci, Staphylococci, *Escherichia coli* and yeast were found to grow in regular chocolate agar and disrupt the culture of *H. pylori*. Yeasts also grew in pylori agar, and only grew in vancomycin-colistin-nystatin (VCN) blood agar as *H. pylori*. We could therefore conclude that VCN would have inhibited the growth of other microbial agents for the benefit of *H. pylori*. Blood agar would be the most suitable medium for the culture of *H. pylori*.

Of the 97 cultures performed, we obtained an overall *Helicobacter pylori* infection rate of 59/79 (60.82%), 79 (81.44%) were positive and 18 (18.56%) were negative ($x^2 = 7.035 > x^2_0 > 3.84$, $p = 0.01$, dof = 1, significant difference in favor of positive cultures).

Of the 79 positive cultures, 59 (75%) of *Helicobacter pylori* isolated and 20 (25.35%) of other isolated microbial agents ($x^2 = 4.291 > x^2_0 > 3.84$, $p = 0.02$, dof = 1, significant difference in favor of *Helicobacter pylori* compared to other microbial agents).

Table 4: Overall result of culture

Microbial agents	n	%
Helicobacter. pylori	59	75
Streptococcus spp	7	9
Streptotoccus agalactiae	4	5,06
Candida albicans	4	5,06
Pseudomonas aeruginosa	2	2,53
Escherichia coli	2	2,53
Staphylococcus aureus	1	1,26
Total	**79**	**100**

n = number, % = percentage

Biochemical identification of H. pylori by urease activity

Part of the biopsy or fecal debris was suspended in 100 µL of urea medium and placed at 37 ° C for 24 hours. In the presence of the bacteria, a colorimetric change from red from phenol to pink is observed (Figure).

Morphological identification of H. pylori by GRAM staining

After the different steps of the GRAM staining, microscopic observation, at the objective x 100 with oil immersion, showed us the morphology of the curved, spiral and pink H. pylori colonies (Photo h, Table 5).

Study of the sensitivity of *H. pylori* isolated from diarrhea and biopsies to antibiotics

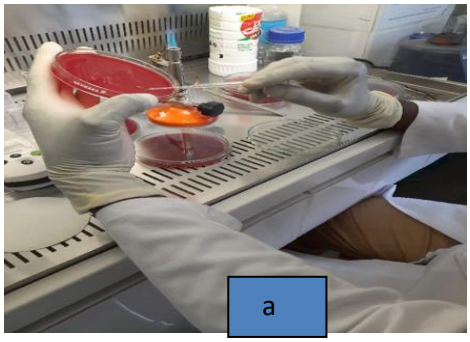
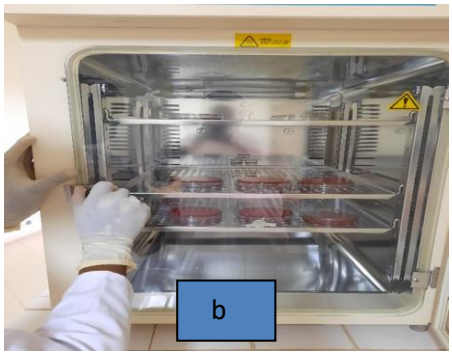
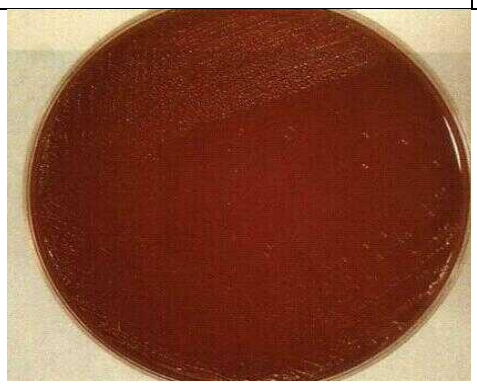
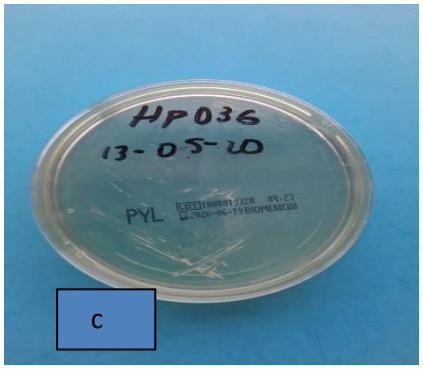
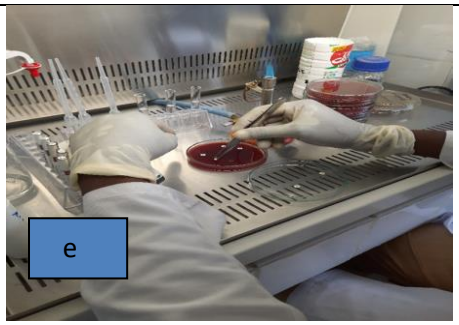
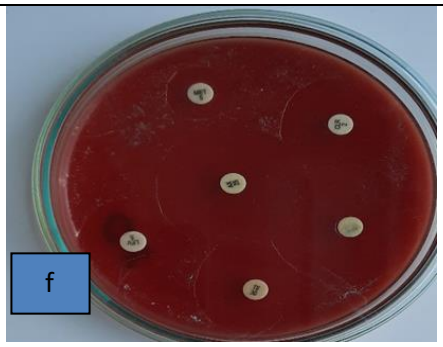
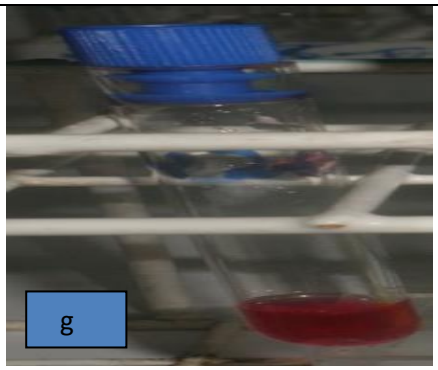
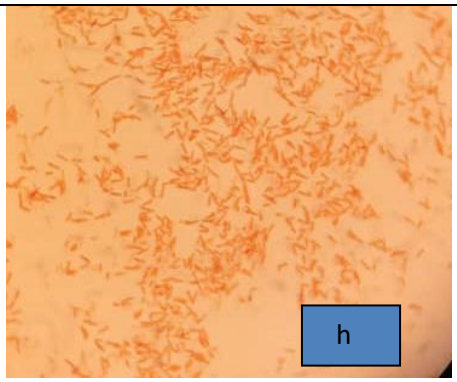
Six antibiotics were tested on the 59 strains of H. pylori isolated. These were clarithromycin, levofloxacin, tetracycline, amoxicillin, amoxicillin + clavulanic acid, and metronidazole. We observed strong resistance to metronidazole. The following table indicates the frequencies of the sensitivities and resistance of the strains to antibiotics.

Table 5: Summary of antibiotics tested on isolated strains

Antibiotiques	n	%	ni	%i	nr	%r
Metronidazole	**9**	**15,25**	**6**	**10,17**	**44**	**74,58**
Clarithromycin	47	79,66	2	3,39	10	16,95
Lévofloxacin	42	71,19	9	15,25	8	13,56
Tétracycline	50	84,75	4	6,78	5	8,47
Amoxicillin	56	94,92	0	0	3	5,08
Amoxicillin + CA	56	94,92	0	0	3	5,08

n: Number of sensitive germs, %: Frequency of sensitive germs, ni: Number of intermediate germs, % i: Frequency of intermediate germs, nr: Number of resistant germs. % r: Frequency of resistant germs.

Table 6: Macroscopic and microscopic characteristics of *Helicobacter pylori* colonies

1	a : seeding b : incubation in a bacteriological oven		
2	c: ***Helicobacter pylori on blood agar. Culture produces clear colonies, which can grow from a gastric biopsy sample (Blood Agar, 6 p.m. a 37 ° C)*** d : ***Helicobacter pylori on H. pylori agar.***		
3	**e** : arrangement of antibiotic discs on blood agar**.** f : *H. pylori* antibiogram.		
4	**g: Positive urease** h : microscopic appearance of *H. pylori* after GRAM staining		

DISCUSSION

Conducted at the National Reference University of N'Djamena, the present study, the first in Chad, consisted in determining the prevalence of *H. pylori* infection as well as the resistance of the bacteria to the antibiotics used in the treatment protocol for the infection.

In this study, we determined a prevalence of *H. pylori* infection of 60.82%. This prevalence was lower than that of Moussa et al, who obtained 83.7% in a study conducted at the General National Reference Hospital of N'Djamena in 2010 [11]. We could explain this gap by the irrational use of antibiotics due to the persistent scale of illicit markets during the last decade in the city of N'Djamena. This prevalence was also higher than that of Naîma which obtained 29% in

Algeria in 2018 [17]. It was similar to that of Doffou et al in Yopougon in Côte d'Ivoire in 2002 [18], to that of Dia et al, in Dakar in Senegal in 2010 [16], to that of Tajeldin et al in Sudan in 2012 **20** who reported prevalence of 57.8%, 65.8% and 65.8% respectively. However, it was lower than that of Firmin et al., In Yaoundé in Cameroon in 2013 [21], that of Bouihat in Morocco in 2017 [22], that of Ahmad in Kano in Nigeria in 2018 [23], and in that of Traoré in Mali in 2020 [24], which reported 72.5%, 69.5%, 81.7%, and 79.60% respectively. It was higher than the prevalence observed in France, fluctuating between 15 and 30% in 2019 [25]. These differences in prevalence could be explained not only by the differences in living standards which are strongly linked to the onset of infection, but also to the misuse of antibiotics and the diagnostic methods used. A 60.82% prevalence of *H. pylori* infection can be explained by the low standard of living of Chadians in the developing world.

In terms of gender, it was observed 60.71% (34/56) in the group of men, and 60.98% (25/41) in the group of women. This result corroborates that of Moussa et al, who reported that gender did not influence the status of patients with *H. pylori* infection [11]. These data were comparable to those of Joutei et al, who obtained 53% for women, 47% for men in Morocco in 2007 [26], and to those of Bouihat who obtained 65.21% in the group of men. and 72.8% in the group of women in Morocco in 2017 [22]. Firmin et al, obtained 78.6% in the group of men and 68.3% in the group of women in Yaoundé, Cameroon in 2013 [21]. Amel et al, had reported a prevalence of 73.2% in men, and 65.5% in women in Morocco in 2013 [27]. In all of these cases, the chi-square test for independence had rejected the hypothesis that the infection was gender-related. This could be explained by the acquisition of the infection which took place mainly in childhood and whose only factors favoring the onset are the immaturity of the gastric mucosa, the low socioeconomic level: hygiene, gastrointestinal enteritis, and promiscuity.

According to the age groups, and in relation to the total infected population, it was observed that the infected patients of the 16 to 39 years old group represented 44.07% (26/59), those of 40 to 63 years old represented 33.90% (20/59), and those aged 64 to 85 accounted for 22.03% (13/59). In relation to their class size, infected patients in the 16 to 39 class represented 65% (26/40), those 40 to 63 years old represented 61% (20/33), and those aged 64 at age 85 had represented 54.17% (20/33).

This result was similar to that of Firmin et al., Who reported 83.1%, 67.4% and 60.8% in the groups under 40, 40 to 50, and over 50 respectively. [21]. It was also similar to that of Doffou et al, who had found a frequency of 57.7% in the age group of 21 to 40 years [18], and that of Traoré who had obtained 48.74% in the age group. 15 to 40 years of age [24]. Moussa et al, reported that age did not influence *H. pylori* infection status [11].

In our study, there was not a significant difference between the prevalences of the first two classes. However, the prevalence of the 64 to 85 age group was slightly lower than that of the first two classes. This could be explained by the progression of the disease with age, the intensification of lesions and symptoms, and therefore some elderly people were previously treated.

Regarding the occupation, the frequencies were between 20 and 25% for all categories. This could be explained by the almost identical standard of living or community habits among all patients despite being from different professions. On the other hand, it could be a social rise for many subjects, as they experienced precarious living conditions in infancy, when the infection occurred. Except students and retirees whose participation in the study was not significant, accounted for 8.47% and 3.39% respectively. This could be explained by the youth of the population, the resort of the population to hospitals often at a very advanced stage of the disease, and the low life expectancy.

Regarding the source, the patients were classified into 3 categories. These were hospital patients, referrals, and outpatient (outpatient) follow-ups. Hospitalized patients dominated with a frequency of 64.41%, followed by patients referred from the various structures of the city which occupied a frequency of 18.64%, and those who are followed externally represented 16.95%. These differences could be explained by the use of the population in hospital often at a very advanced stage of the disease and therefore they occur when their condition requires hospitalization. Most of the hospitalized patients first went through the emergency department of the CHU-RN.

Self-medication, especially with antibiotics, has a crucial role in the progression of the disease, the development of resistance and the reliability of the microbiological result. During the interview, 23.73% admitted that they were used to self-medication, 55.93% said they were not used to self-medication, and 20.34% had ignored. Doffou et al., Had reported 17.3%, 9.6% and 12.6% having taken a gastrotoxic drug, an antibiotic or a gastric antisecretory respectively the 4 weeks preceding the endoscopic examination in Yopougon in Côte d'Ivoire in 2002 [18].

Speaking of the reason for the endoscopy, and compared to the infected population, patients who consulted for epigastralgia dominated with a frequency of 54.24%, followed by Others (Nausea, vomiting, hematemesis, regurgitation, etc.) with 23.73%, heartburn at 15.25%, and dyspepsia at 6.78%. These data were comparable to those of Ontsira who reported 64.3% for epigastralgia, 6.3% for dyspepsia, and 5.6% for heartburn in Congo Brazzaville in 2016 [19, 28]. They were similar to those of Doffou et al., Who had shown epigastralgia as the main reason for endoscopic examination with a frequency of 82.7% in Yopougon in Côte d'Ivoire in 2002 [18].

According to the endoscopy result, the study found gastritis 88.14%, esophagitis (more often peptic) 83.05%, gastric ulcer 57.63%, ulcer duodenal at 45.76%, varicose veins at 11.86%, the appearance of normal mucosa at 8.47%, and pseudo-nodular gastropathy at 10.17%. These data were not comparable to those of Moussa at al., Those of Ontsira, those of Firmin et al., That of Emal et al., And that of Naïma. Moussa et al., Reported 7.6% esophagitis, 2.3% gastric ulcer, and 19.8% duodenal ulcer in N'Djamena in 2010 [11]. Ontsira had obtained 36.4% of normal mucosa, 22.4% of gastropathy, and 17.4% of peptic ulcer disease in Congo Brazzaville in 2016 [28]. Firmin et al., Found that the main lesions among patients were antral gastritis at 49.7%, diffuse gastritis at 29.8%, duodenal ulcer at 15.8%, peptic esophagitis at 11, 1%, gastric cancer at 1.2%, and the examination was normal at 7.6% in Yaoundé, Cameroon in 2013 [21]. Emal et al., Reported 91.8% of chronic gastritis in infected patients, 5% of gastric ulcer and 3.2% of gastric cancer in Morocco in 2013 [27]. Naïma had reported in patients who had not undergone previous treatment, 81.6% of gastritis, followed by duodenal ulcers at 10.45%, and gastric ulcers at 3.26%, lymphomas at 0.65%. while 3.92% of them had a normal gastric mucosa in Algeria in 2018 [17]. However, they remained comparable to those of Dia et al., Who had shown chronic gastritis at 60.6%, duodenal ulcer at 77% and gastric ulcer at 75% in Dakar, Senegal in 2010 [16].

The course of these cascades of lesions depends on the duration of the infection, and the presence of other contributing or triggering factors such as taking NSAIDs for ulcers. In this study, we did not record any cases of cancer. The high prevalence of gastric and duodenal ulcers could be explained by patients going to hospital at very advanced stages of the infection.

As we mentioned, the yeasts had grown in GC and G. Pylori. *Pseudomonas*, Streptococci, Staphylococci and *Escherichia coli* have grown in GC.

We could explain the presence of germs other than *H. pylori* by a supply of these germs in the stomach from the oral cavity and the duodenum, and by contamination during the passage of the endoscopic probe. We used the three environments in order to reassure ourselves of their reliability and to have a good result.

Regarding antibiotic resistance, that to clarithromycin is characterized by the decrease in the affinity of the antibiotic for its target and confers on the bacteria cross resistance to macrolides. We detected a sensitivity rate of 79.66% and a resistance rate of 16.95%. This frequency was not comparable to those of other series reported such as 0% by Secka et al, in The Gambia in 2013 [2], 0% by Oyedeji et al., In Nigeria in 2009 [3], 0% by Asrat et al., In Ethiopia in 2004 [4], 1% by Seck et al., In Senegal in 2013 [28], 1.7% by Ontsira in Congo Brazzaville in 2016 [28], 6.4% by Kimang in Kenya in 2010 [29]. However, was similar to that of Ben Mansour et al., Who reported 15.4% in Tunisia in 2010 [15], to that of Tanih et al., Who obtained 20% in South Africa in 2013 [30]. It was much lower than that of Raymond et al., Who found 26% in France in 2010 [31], that of Bouihat who reported 28.6% in Morocco in 2017 [22], and that of Djennane- Hadibi et al., Who obtained 33% in Algeria in 2016 [32]. In 2010, primary resistance to clarithromycin was 17.2% worldwide. It would be related to its prescription in respiratory infections when an atypical germ is suspected [16].

Clavulanic acid helps overcome resistance due to beta-lactamase. With Amoxicillin + AC, we achieved the same result as with amoxicillin. This allowed us to confirm the absence of beta-lactamase in *H. pylori*. We determined a susceptibility rate of 94.92% and a resistance rate of 5.08% to amoxicillin. This resistance rate was different from those of Bouihat in Morocco in 2017 [22], Dia et al. in Dakar in 2008 [19], and Naïma in 2018 in Algeria [17], who had not obtained resistance to amoxicillin. The CNRCH reported in its 2019 activity report a resistance of 2% in France [33].

Amoxicillin resistance is rare, and given its low frequency, CASFM does not recommend its testing [17]. The frequency of resistance to amoxicillin observed in our study is among the strongest resistance of *H. pylori* to this antibiotic in the world. This could be explained by the irrational overuse of this antibiotic. Unlike clarithromycin, Levofloxacin and tetracycline, amoxicillin remains the most accessible antibiotic known and used in the Chadian community.

Levofloxacin resistance is believed to be linked to the occurrence of point mutations in a particular region of the gyr A subunit of the target DNA gyrase of fluoroquinolones. We determined a sensitivity rate of 71.19% and a resistance rate of 13.56% to levofloxacin. This resistance rate was superimposed on that of Bouihat and Kalach, but higher than that of Naïma and Ontsira. Bouihat had obtained a resistance rate of 10.7% to levofloxacin in Morocco in 2017 [22], Kalach had found 13.2% in France in 2007 [14], Ontsira had obtained 1.2% in Congo Brazzaville in 2016 [28], and Naïma reported 2% in Algeria in 2018 [17]. The CNRCH had reported in its annual activity report for 2019 a resistance rate of 19.1% for Levofloxacin in France [33]. Globally, De Francesco et al. Reported in 2010 a frequency of 16.2% resistance to fluoroquinolones in H. pylori, [16]. These high rates of resistance could be explained by the use of levofloxacin in communities because of its prescription for urogenital and ENT infections.

The efflux is believed to be implicated in the occurrence of resistance in *H. pylori* to tetracycline, but mutations are also responsible for this resistance. We determined a susceptibility rate of 84.75% and a resistance rate of 8.47% to tetracycline. In 2010, De Francesco et al., Found a frequency of 5.9% in the world with a great disparity between Africa where it reached 43.9% [16]. Ontsira et al., Had reported a resistance of 4.2% [28]. The CNRCH in France in 2019, Naïma in 2018 in Algeria, and Bouihat in 2017 in Morocco did not find resistance to tetracycline.

Resistance to metronidazole affects several genes. It was on average 3 times higher than for macrolides worldwide in 2014 [9]. In this study, we determined a sensitivity rate of 15.25% and a resistance rate of 74.58%. This resistance rate was higher than that of Bouihat in Morocco in 2017 who had reported 40.1%, and that of Ben Mansour et al, who had obtained 56% in Tunisia in 2010 [15]. It was lower than that of Seck et al, who found 85% in 2013 in Senegal [13]. In Europe, it was 34.9% in adults and 25.7% in children in 2007 [14].

This strong resistance can be explained not only by the strong prescription of this antiparasitic antibiotic in many parasitosis, whether as a preventive or curative measure, but also by the irrational use of this drug which is known to all.

CONCLUSION

Although localized, this study determined a high prevalence of *H. pylori* infection in the city of N'Djamena. This constitutes a real public health problem for Chad. The occurrence of infection does not depend on gender or age, let alone socioeconomic status. With regard to antibiotic resistance, these results suggest the need for the implementation of management procedures guided by the results of the antibiogram.

Finally, we recommend that large-scale studies be carried out in the provinces in order to better understand the extent of the resistance of *H. pylori* to the various antibiotics used for its eradication.

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Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical and Administrative Considerations: Our study previously received:

- Authorization from the Dean of the Faculty of Human Health Sciences (FSSH), University of N'Djamena (Chad);

- Authorization from the Director General of the National Reference General University Hospital (CHIRN) of N'Djamena (Chad);

- Verbal consent of each patient or his beneficiary to whom we have explained the procedure and the importance of the study and their participation.

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