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# Diagnosis of helicobacter *pylori* infection in low out-outcome country: Rapid urease test, serological test, versus direct microbiological examination with gram staining

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**Keywords:** Helicobacter pylori; Diagnosis tests; Sensitivity; Specificity; Positive and negative predictive value.

**Abbreviations:** H. pylori: Helicobacter pylori; RUT: Rapid Urea Test; NPV: Negative Predictive Value; PPV: Positive Predictive Value.

## Abstract

**Introduction**: *Helicobacter pylori* is a Gram-negative bacillus, responsible for numerous gastroduodenal pathologies and this infection constitutes a public health problem. The prevalence of infection with this bacterium remains high in countries with limited resources. Its diagnosis is mainly based on many indirect methods (urease test and serological test). The objective of this work was to evaluate the various indirect methods of diagnosis regarding bacterial culture.

**Methodology**: We conducted a cross-sectional and analytical study from January to May 2022 in the Gastroenterology departments of the Douala General Hospital and the Douala Military Hospital. All patients aged 18 years and above, at the gastroenterology consultation and who agreed to participate were included in our study. Sociodemographic, clinical and paraclinical data were collected. Urease, liquid urea and culture tests were made from samples obtained by fibroscopy. Serological tests were carried out with to the blood sample.

**Results**: Among the 101 patients included, 58 were females and 43 were males with a sex ratio of 1.3. The mean age was 44.2 ± 16 years. The prevalence of infection was 90.5%, 44.1%, 40.6% and 21.8% respectively for serology, direct microbiological examination, RUT, and culture. Comparing different tests, sensitivity and specificity were respectively 67.1% and 64% for the RUT, 100% and 73.7% for direct microbiological examination, 100% and 14.8% for serology. Positive and negative predictive values were respectively, 39.5% and 100% for serology, 39% and 85% for the RUT, 55.6% and 100% for direct microbiological examination.

**Conclusion:** The prevalence of *Helicobacter pylori* infection depend on the type of test used. Direct examination present better reliability than RUT and serology.

## Introduction

*Helicobacter pylori* is a Gram-negative bacillus, known as the most common bacterial infection [1]. The transmission is essentially interhuman and occurs via the faecal-oral, oro-oral or gastro-oral routes, but also iatrogenically through gastric intubation [2-4]. The primary infection with *Helicobacter pylori* occurs mainly in childhood and favored by promiscuity and low socioeconomic status [4]. In the long term, colonization by *Helicobacter pylori* can damage the gastric mucosa and cause various diseases of the gastrointestinal tract like gastritis, Peptic Ulcer Disease (PUD) and gastric cancer (adenocarcinoma and lymphoma) [3,5]. Its prevalence is estimated at 50% worldwide, where 70-80% of cases are from resource-limited countries and 15-30% from industrialized countries [6,7]. In Cameroon, the overall prevalence of H. pylori infection varies from 47.4% to 72.5% according to the studies carried out [8-11]. This variation is related to the different techniques used in these studies for the diagnosis of H. pylori infection. Several methods invasive and non-invasive have been developed and validated for the diagnosis of H. pylori [7,12-14]. Among the non-invasive methods, there is the serology based on the search for Ig G antibodies, the stool antigen test, the urea breath test [12]. Invasive methods require the realization of an oesogastroduonal endocopy during which biopsies are performed for analysis. These methods are the rapid urease test done in endoscopy room, culture, molecar based testing and histology [12]. Invasive tests require rigorous pre-analysis conditions for conservation and transport of samples that are little or poorly applied in current practice [14]. In addition, the invasiveness of the endoscopy contributes strongly to the use of non-invasive tests. Although highly sensitive and specific, non-invasive tests often require additional testing to confirm the diagnosis, as is the case with serology. In the African study to determine the prevalence of H. pylori infection, we find that the diagnostic methods used differ from one series to another, which contributes to a large variation in the results obtained [8,10,11,15]. Majority of gastroenterologists prefer the rapid test to urease which is easy to access, or the pathological examination which unfortunately very expensive, as recommended for the diagnosis of *H. pylori* infection [13,16]. Serology is often tested by other specialists or general practitioners. Microbiological examination with Gram staining although available and easy to access is very little used because unknown to many gastroenterologists. The purpose of the study was to compare tests commonly used for the diagnosis of *H. pylori* (serological test, Rapid urea test) versus direct microbiological examination; and thus determine their sensitivity, their specificity and predictive values.

## Materials and methods

### Type of study

We conducted a cross-sectional study over a period of 06 months from January 1 to June 30, 2022. It had as framework 02 hospitals of the city of Douala the general hospital and the military hospital, which have a service of digestive endoscopy. We included any patient who was at least 18 years old and received in the digestive endoscopy unit for an oesogastroduodenal endoscopy and consented to participate in the study. We excluded patients with tumor-like lesions, those who had taken antibiotics (amoxicillin, clarithromycin, metronidazole, levofloxacin) and/or a proton pump inhibitor in the month prior to study inclusion date. The Institutional Ethics Committee for Research on Human health of the University of Douala, approved the study and all subjects gave written informed consent before participation.

### Sampling procedure

Each patient received from the principal investigator an explanatory sheet of the study supplemented with oral explanations. An informed consent sheet was provided to the patient for signature after the patient verbally consented to study participation. A pre-established, anonymous data collection form for each patient was completed by the investigator. the data collected were socio-demographic data (age, sex), history and comorbidities (high blood pressure, diabetes, HIV, H. pylori anterior infection), clinical signs presented by the patients (epigastralgia, dyspepsia, regurgitation, pyrosis, nausea, vomiting). Concerning blood sample, 3 ml of venous blood was collected using, a vacutainer needle and a dry tube for serologic analysis. During the oesogastroduodenal endoscopy performed by the gastroenterologist, biopsies were done for the microbiological examination in the following way: 02 at the antrum, 02 in the fundus and 01 at the angle of the small curvature. These biopsies were used to perform the liquid urea stain and culture test. Two additional biopsies were done at the antrum to perfom rapid urea test. During the procedure, biopsy specimens taken with biopsy pliers were inserted into pre-labelled sterile urine boxes (anonymity, patient sex and age), containing 3ml of brain heart broth and 20% glycerol (transport medium). These samples were sent to the site of operation using a cooler containing dry ice. These samples were stored in the refrigerator (4-8°C) for 24 hours or -60°C beyond 24 hours.

## Analytical steps

### Serological test

The serological test was carried out using the antibodies directed against *H. pylori* by enzyme-linked immunosorbent assay from the Diaspot kit. Two drops of serum samples and one drop of buffer were inserted into the cassette well from a vertical dropper. After 10 minutes of migration, the positivity of the test is determined by the presence of two bars.

### Rapid urease Test

Biopsies were deposited in the well containing an acid pH urea solution with a yellow coloured marker (phenolphthalein). The result was read 5-30 minutes after the biopsy was deposited. The test was positive if the disc circumference turned pink. The intensity of the red discolouration of the disc depends on the density of the population of *Helicobacter pylori* at the sampling site.

### Direct microbiological examination and Culture

Once arrived at the laboratory, the biopsies were immediately crushed with a pestle in a mortar containing a few drops of heart-brain broth to facilitate the crushing [17]. At the end of this stage, each mill underwent two further treatments: direct micobiological examination and culture.

### Direct microbiological examination with gram staining

A small amount of the crusher was placed on a clean blade and spread by circular movements. After drying the blade at room temperature, he followed the staining by the Gram Method and finally the observation of the dried smear was added to a drop of immersion oil microscopically to objective 100. The observation of a spiral-shaped bacillus, 2-4 µm long and 0.5-1 µm wide, coloured pink (Gram-) indicates the presence of *Helicobacter pylori*.

The principle of gram staining is based on the staining of the bacterial cell wall. It has four stages including, staining the smear with gentian violet (30 seconds to 1 minute), etching with Lugol (30 seconds to 1 minute), discoloration of the slide with alcohol (30 seconds) and counter-staining with Fuchsine (30 seconds to 1 minute).

### Culture

We added 47 g of Columbia agar (powder) to 1 L of distilled water and the mixture was heated until fully cooked. The bottled mixture was then autoclaved for 15 minutes (to eliminate bacteria that could not be destroyed at high temperatures). At 45°C temperature, 10% of human blood added to the mixture was homogenized. The mixture obtained was finally added to an OXOID brand supplement comprising vancomycin (10 mg/l), trimethoprim (05 mg/l), cefsulodin (10 mg/l) and amphotericin B (10 mg/l). l), then poured into Petri dishes. A small quantity of the biopsy homogenate was streaked on the culture medium and then incubated at 37°C in the absence of oxygen for a maximum of 10 days. The incubated culture dishes were examined every 24 hours. It was not until the 10th day of incubation with no visible suspicious colonies that the culture was considered sterile. Suspicious colonies (small colonies of about 0.5 to 1 mm in diameter, translucent, shiny and non-haemolytic) isolated, were subjected to morphological and biochemical identification tests.

**Morphological identification:** was carried out by the gram of control, which consists in carrying out a Gram staining of the suspicious colonies spread out on a slide (refer to direct microbiological examination above).

•**Biochemical identification:** consists of performing a catalase test, an oxidase test and a urea-indole test.

- **Catalase test** is done by adding a drop of hydrogen peroxide to a slide previously containing a colony of isolated bacteria. The positivity of the test is marked by the appearance of gas bubbles on the slide.

- **Oxidase test** involves bringing a suspect colony of *Helicobacter pylori* into contact with an oxidase disk. The positive reaction is marked by the colour change of the disc to purple.

- **Urea-indole test** is performed by adding a suspect colony to an Eppendorf tube containing a small amount of indole urea. The change in colour from yellow to pink after 24 hours marks the positivity of the test.

### Statistical analyzes

Data were analyzed with SPSS version 26.0 sotfware. Dichotomized data were used to calculate sensitivity, specificity, Positive Predictive Value (PPV), negative predictive value (NPV). The values are given with 95% confidence interval. Each test was test against culture as gold standard.

## Results

We included 101 patients with gastroduodenal symptoms were consecutively received in the endoscopy department. The mean age was 44.2 ± 16 years with median equal to 44 years. We had 58 women and 43 men, a sex ratio of 1.3 (Table 1). High blood pressure was found in 13 patients (12.9%) and diabetes in 5 patients (4.9%). In terms of lifestyle, 88.1% (n=89) had a consumption of spices, 54.5% (n=55) had a consumption of alcohol and 7.9% (n=8) had a notion of smoking (Table 1). For oesopgastroduodenal endoscopy, lesions were found in 73.3% of patients (n=74). The three main endoscopic lesions were erythematous antral gastropathy (57.4%), bulbar ulcer (14.9%), and pangastropathy (13.9%) (Table 1). The prevalence of *Helicobacter pylori* infection was 90.5% for the serological test, 57.4% for the liquid urea test, 44.1% for direct microbiological examination, 40.6% for the rapid urease test, and 21.8% for culture. Comparing the different diagnostic tests, sensitivity and specificity were 64% (IC95% 52.5-73.6) and 67.1% (IC95%: 48.6-78.5) respectively for the rapid urease test, 100% (IC95%: 95.6-100) and 73.7% (IC95% 68.7-88.4) for direct microbiological examination, 100% IC95% 98-100) and 14.8% (IC95% 8.3-17.9) for serology (Table 2). Positive predictive values were 39.5% (IC95% 12.7-83.2) for serology, 39% (IC95% 41.6–78.4) for the rapid urease test, 55.6% (IC95% 52.3-77.9) for direct microbiological examination (Table 2). Negative predictive values were 100% (IC95% 98-100) for serology, 85% (IC95% 83.4-92.7) for the rapid urease test, 100% (IC95% 97-100) for direct microbiological (Table 2).

**Table 1:** Population of study.

| | Frequence (%) | Mean (SD) |
|---|---|---|
| **Age (years)** | | 44.2(16) |
| **Sex** | | |
| **Men** | 48 (47.5) | |
| **Women** | 53 (52.5) | |
| **Comorbidities ang Lifestyle** | | |
| **Hypertension** | 13 (12.9) | |
| **Diabetes** | 5 (4.9) | |
| **Alcohol** | 55 (54.5) | |
| **Tobacco** | 8 (7.9) | |
| **Spicy food** | 89 (88.1) | |
| **Clinical presentation** | | |
| **Epigastralgia** | 76 (75.3) | |
| **GERD** | 60 (59.4) | |
| **Dyspepsia** | 48 (47.5) | |
| **Loss of weight** | 45 (44.6) | |
| **Nausea** | 41 (40.6) | |
| **Endoscopic features** | | |
| **Normal** | 27 (26.7) | |
| **erythematous antral gastritis** | 58 (57.4) | |
| **Bulbar ulcer** | 15 (14.9) | |
| **Pangastritis** | 14 (13.9) | |

**Table 2:** Sensitivy, specifity and predictives values of RUT, serology and direct micorbiological examination according to culture.

| | Sensitivity (IC95%) | Specifity (IC95%) | PPV (IC95%) | NPV (IC95%) |
|---|---|---|---|---|
| **RUT** | 64% (52.5-73.6) | 67.1% 48.6-78.5) | 39% (41.6–78.4) | 85% (83.4-92.7) |
| **Direct Examination** | 100% (95.6-100) | 73.7% (68.7-88.4) | 55.6% (52.3-77.9) | 100% (97-100) |
| **Serology** | 100% (98-100) | 14.8% (8.3-17.9) | 39.5% (12.7-83.2) | 100% (98-100) |

## Discussion

The prevalence of H pylori infection depended on the diagnostic test used. With regard to the rapid urease test, direct microbiological examination the prevalences found were similar to those found in various studies in Cameroon but also in other countries of sub-Saharan Africa [8,10,11,18,19]. There are a few studies in Cameroon with higher prevalences, for which the type of diagnostic tests used and the target population must be taken into account [9]. The high prevalence found for the serological test is probably related to the fact that the latter is based on the search for Ig G antibodies [20]. It is therefore difficult to associate with an active infection. Concerning culture, the low prevalence found, which contrasts strongly with the available data in terms of prevalence in Africa [18] and Cameroon, is related to the technical constraints encountered [17]. Indeed, the conditions for the realization of the culture are sricts and often difficult to implement in current practice.

Although the culture for the diagnosis of *H. pylori* infection presents technical limitations and low prevalence in this series, we wanted to use it as standard gold to compare diagnosis methods because it presents in the literature a good specificity with a correct sensitivity [16,21].

The sensitivity of the serology was good as described in the literature as well as that of the direct microbiological examination. However, the sensitivity of the rapid urease test was below those found in various studies. This result raises questions about the quality of the kits available and used for the rapid urease test. The specificity was very low for serology, because the Ig G can be found even after eradication of the bacteria [16]. There is also a good specificity of direct microbiological examination compared to the rapid urease test.

Concerning predictive values, the direct microbiological examination and serological test had a good negative predictive value that was significantly higher than the rapid urease test. All the test we used had low positive predictive value. The values were less than 60%. The direct microbiological examination had the better positive predictive values. The results obtained for the rapid urease test are in contradiction with those described in many studies [21,22]. Redeen et al showed the results more than 90% for sensitivity, specifity and predictives values for RUT when the biospies were performed in the antrum [22]. We did the biopsies for RUT in the antrum. The reliability of the kits and the time taken to read the results could be decisive factors on perfomances of the rapid urease test. Van Horn et al, in 1990 showed a better sensitivity and specificity when the kit is reviewed 24 hours later [23].

The results obtained with the direct microbiological examination with Gram staining open the door to its use in current practice, as Oyedeji et al had already mentioned in Nigeria [24]. He had found a higher prevalence in use direct examination with Gram staining compared to culture and respiratory testing.

The main limitations of the studies were the lack of sufficient comparative data on the reliability of the direct microbiological test with Gram staining, particularly with the pathological test and stool antigen test.

## Conclusion

The prevalence of H. pylori infection depends on the type of diagnostic tests used. The prevalences found with the urease test and with direct microbiological examination are similar to those described in the literature in Cameroon. Direct microbiological examination showed good results in terms of sensitivity and specifivity as well as good predictive values. It could be an alternative to pathological examination which is costier. The rapid urease test, although having a lower sensitivity and specificity compared to that found in the various studies, still retains a good negative predictive value. It remains a more reliable test than the serological test

## Declarations

**Conflict of interest statement:** The authors state that they have no conflict of interest.

**Author's contrbutions:** Data collection and analysis: Winnie Bekolo and Ilinga Kelly.

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