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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 Stain

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Abstract

Introduction: *Helicobacter pylori* is a gram-negative bacillus responsible for numerous gastroduodenal pathologies, and this infection is a public health problem. The prevalence of infection with this bacterium remains high in countries with limited resources. Diagnosis relies mainly on numerous invasive and noninvasive methods. The aim of this work was to evaluate the different indirect diagnostic methods using bacterial cultures. **Methods:** We conducted a cross-sectional and analytical study from January to May 2022 in the gastroenterology departments of Douala General Hospital and Douala Military Hospital. All patients aged 18 years and older who were in the gastroenterology consultation and agreed to participate were included in our study. Sociodemographic, clinical, and paraclinical data were collected. Urease, liquid urea, and culture tests were performed from the specimens obtained by fibroscopy. Serological tests were performed on the blood sample. **Results:** 101 patients were included, 58 were female and 43 were male, for 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% for serology, direct microbiological examination, RUT (rapid urea test) and culture, respectively. Comparison of the different tests showed sensitivity and specificity of 67.1% and 64%, respectively, for RUT, 100% and 73.7%, respectively, for direct microbiological

examination, and 100% and 14.8%, respectively, for serology. The positive and negative predictive values were 39.5% and 100% for serology, 39% and 85% for RUT, and 55.6% and 100% for direct microbiological examination, respectively. **Conclusion:** The prevalence of *Helicobacter pylori* infection depends on the type of test used. Direct examination is more reliable than RUT and serology.

Keywords

Helicobacter pylori, Diagnostic Tests, Sensitivity, Specificity, Positive and Negative Predictive Value

1. Introduction

Helicobacter pylori is a gram-negative bacillus, that is considered the most common bacterial infection [1] [2]. Transmission is mainly interpersonal via the fecal-oral, oro-oral or gastro-oral route, but also iatrogenic by gastric intubation [2] [3] [4]. Primary infection with *Helicobacter pylori* occurs mainly in childhood and is favored by promiscuity and low socioeconomic status [4]. In the long term, colonization with *Helicobacter pylori* can damage the gastric mucosa and cause various gastrointestinal diseases such as gastritis, peptic ulcer disease (PUD), and gastric cancer (adenocarcinoma and lymphoma) [3] [5]. The prevalence is estimated at 50% worldwide, with 70% - 80% of cases from resource-limited countries and 15% - 30% from developed countries [6] [7]. In Cameroon, the overall prevalence of *H. pylori* infection varies from 47.4% to 72.5% depending on the study [8] [9] [10] [11], which, is due to the different techniques used to diagnose *H. pylori* infection. Several invasive and non-invasive methods have been developed and validated for the diagnosis of *H. pylori* [7] [12] [13] [14]. Noninvasive methods include serology based on the search for IgG antibodies, stool antigen test, and the urea breath test [12]. Invasive methods require the performance of esogastroduonal endoscopy, in which biopsies are taken for analysis. These methods are the rapid urease test performed in the endoscopy room, cultures, molecular testing and histology [12]. Invasive tests require strict pre-analysis conditions for the preservation and transport of prior which are rarely or poorly applied in current practice [14]. In addition, the invasiveness of endoscopy strongly contributes to the use of noninvasive tests. Although the noninvasive tests are very sensitive and specific, they often require additional tests to confirm the diagnosis, as is the case with serology. In the African study to determine the prevalence of *H. pylori* infection, it was found that the diagnostic methods used varied from one series to another, contributing to a wide variation in the results obtained [8] [10] [11] [15]. Most gastroenterologists prefer the readily available rapid urease test, or the unfortunately very expensive pathological examination, as recommended for the diagnosis of *H. pylori* infection [13] [16]. Serology is often tested by other specialists or general practitioners. Microbiological test

with Gram stain although available and readily easy is very underutilized, because unknown to many gastroenterologists. The aim of the study was to compare tests commonly used for the diagnosis of *H. pylori* (serological test, rapid urea test) versus direct microbiological test; and thus determine sensitivity, specificity and predictive value.

2. Methods

2.1. Type of Study

We conducted a cross-sectional study over a 6-month period, from January 1 to June 30, 2022. The setting was 02 hospitals in Douala City, the general hospital and the military hospital, which have a digestive endoscopy service. All patients who were at least 18 years old and admitted to the digestive endoscopy service for esogastroduodenal endoscopy and agreed to participate in the study were included in the study. Patients with tumor-like lesions and those who had taken antibiotics (amoxicillin, clarithromycin, metronidazole, levofloxacin) and/or a proton pump inhibitor in the month before study entry were excluded. The University of Douala Human Research Ethics Committee approved the study, and all subjects provided written informed consent prior to participation.

2.2. Sampling Procedure

Each patient received an information sheet about the study from the principal investigator, supplemented by verbal explanations. After the patient gave verbal consent to participate in the study, he or she was presented with an informed consent form for signature. The investigator completed a prepared anonymous data collection form for each patient. Sociodemographic data (age, sex), medical history and concomitant diseases (hypertension, diabetes, HIV, *H. pylori* anterior wall infection), clinical symptoms of the patients (epigastralgia, dyspepsia, regurgitation, pyrosis, nausea, vomiting) were recorded. For blood collection, 3 ml of venous blood was drawn with a vacutainer needle and a dry tube for serological analysis. During esogastroduodenal endoscopy performed by the gastroenterologist, the following biopsies were taken for microbiological analysis: 02 at the antrum, 02 at the fundus, and 01 at the angle of the lesser curvature. These biopsies were used to perform the liquid urea stain and culture test. Two additional biopsies were taken from the antrum to perform a rapid urea test. During the procedure, biopsy specimens collected with biopsy forceps were placed in prelabeled sterile urine boxes (anonymity, sex, and age of patient) containing 3 ml of brain heart broth and 20% glycerol (transport medium). These samples were transported to the site in a cool box with dry ice. Samples were stored in the refrigerator (4˚C - 8˚C) for 24 hours or at −60˚C beyond 24 hours.

3. Analytical Steps

1) Serological test

Serological assay was performed with the antibodies directed against *H. pylori*

from the Diaspot kit using an enzymatic immunosorbent assay. Two drops of serum samples and one drop of buffer were added to the well of the cassette using a vertical dropper. After 10 minutes of migration, the positivity of the assay is determined by the presence of two bars.

2) Rapid urease test

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

3) Direct microbiological examination and culture [17]

Once in the laboratory, the biopsies were immediately crushed with a pestle in a mortar that contained a few drops of heart-brain broth to facilitate the crushing. At the end of this phase, each grinder was subjected to two further treatments: direct microbiological examination and culture.

❖ **Direct microbiological examination with Gram stain**

A small amount of the breaker was applied to a clean blade and spread with circular movements. Drying of the blade at room temperature was followed by staining by the Gram method and finally observation of the dried smear in a drop of immersion oil microscopically with objective 100. Observation of a spiral-shaped, 2 - 4 µm long and 0.5 - 1 µm wide, pink (Gram) stained bacillus indicates the presence of *helicobacter pylori*.

The principle of Gram staining is based on the staining of the bacterial cell wall. It consists of four steps: Staining of the smear with gentian violet (30 seconds to 1 minute), etching with Lugol (30 seconds to 1 minute), staining of the slide with alcohol (30 seconds), and counterstaining with fuchsin (30 seconds to 1 minute).

❖ **Culture**

We added 47 g of Columbia agar (powder) to 1 l of distilled water and heated the mixture until it was fully boiled. The bottled mixture was then autoclaved for 15 min (to eliminate bacteria that could not be killed at high temperatures). At a temperature of 45˚C, 10% of the human blood added to the mixture was homogenized. Finally, the obtained mixture was mixed with an OXOID brand preparation containing vancomycin (10 mg/l), trimethoprim (05 mg/l), cefsulodin (10 mg/l) and amphotericin B (10 mg/l), and then poured into Petri dishes. A small amount of the biopsy homogenate was sprinkled 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. Only after the 10th day of incubation, when no suspicious colonies were visible, was the culture considered sterile. The isolated suspicious colonies (small colonies about 0.5 to 1 mm in diameter, translucent, shiny, and nonhemolytic) were subjected to morphological and biochemical identification tests.

Morphological identification: was performed by the Gram control, which

consists in performing a Gram stain on the suspicious colonies spread on a slide (see direct microbiological examination above).

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

- **Catalase test** is performed by placing a drop of hydrogen peroxide on a slide that previously contained a colony of isolated bacteria. Positivity of the test is indicated by the appearance of gas bubbles on the slide.
- **Oxidase test** consists of contacting a suspected colony of *Helicobacter pylori* with an oxidase disc. The positive reaction is indicated by a purple coloration of the disc.
- **Urea-indole test** is performed by placing a suspect colony in an Eppendorf tube containing a small amount of indole urea. The color change from yellow to pink after 24 hours indicates that the test is positive.

Statistical analysis

Data were analyzed using SPSS version 26.0 software. The dichotomized data were used to calculate sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Values are reported with a 95% confidence interval. Each assay was tested against a culture as a gold standard.

4. Results

We included 101 patients with gastroduodenal symptoms consecutively admitted to the endoscopy department. As shown in Table 1, the mean age was 44.2 ± 16 years, with a median age of 44 years. We had 58 women and 43 men, corresponding to a sex ratio of 1.3. Hypertension was detected in 13 patients (12.9%) and diabetes in 5 patients (4.9%). Regarding lifestyle, 88.1% (n = 89) consumed spices, 54.5% (n = 55) consumed alcohol, and 7.9% (n = 8) smoked (Table 1). Oesopgastroduodenal endoscopy revealed lesions 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 rapid urease test, and 21.8% for culture. Table 2 shows that the sensitivity and specificity of the different diagnostic tests are 64% (IC 95% 52.5 - 73.6) and 67.1% (IC 95%: 48.6 - 78.5), respectively, for the rapid urease test, 100% (IC 95%: 95.6 - 100) and 73.7% (IC 95% 68.7 - 88.4), respectively, for the direct microbiological examination, and 100% (IC 95% 98 - 100) and 14.8% (IC 95% 8.3 - 17.9), respectively, for serology. The positive predictive values were 39.5% (IC 95% 12.7 - 83.2) for serology, 39% (IC 95% 41.6 - 78.4) for the rapid urease test, and 55.6% (IC 95% 52.3 - 77.9) for the direct microbiological examination (Table 2). As can be seen in Table 2, the negative predictive values were 100% (IC 95% 98 - 100) for serology, 85% (IC 95% 83.4 - 92.7) for the rapid urease test, 100% (IC 95% 97 - 100) for the direct microbiological examination.

Table 1. Population of study.

	Frequence (%)	Mean (SD)
Age (years)		44.2 (16)
Sex		
Men	48 (47.5)	
Women	53 (52.5)	
Comorbidities and 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 (IC 95%)	Specifity (IC 95%)	PPV (IC 95%)	NPV (IC 95%)
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)

PPN: Positive Predictive Value, NPV: Negative Predictive Value.

5. Discussion

The prevalence of *H. pylori* infection depended on the diagnostic test used. For

the rapid urease test, the direct microbiological test, the prevalences found were comparable to those found in various studies in Cameroon as well as in other countries in sub-Saharan Africa [8] [10] [11] [18] [19]. There are a few studies in Cameroon with higher prevalences, but the type of diagnostic tests used and the target population must be considered [9]. The high prevalence found for the serological test is probably related to the fact that it is based on the search for Ig G antibodies [20]. It is therefore difficult to associate it with active infection. Regarding culture, the low prevalence, which contrasts strongly with available data on prevalence in Africa [18] and Cameroon, is related to technical constraints [17]. Indeed, the conditions for performing culture are often difficult to implement in current practice.

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

The sensitivity of serology was good as reported in the literature as was that of direct microbiological testing. However, the sensitivity of the rapid urease test (RUT) was lower than that reported in various studies. Specificity was low with serology, Ig G can be found even after eradication of the bacteria [16]. Direct microbiological examination also has a good specificity compared to the rapid urease test. As for the predictive values, the direct microbiological test and the serological test had a good negative predictive value, which was significantly higher than the rapid urease test. All the tests we used had a low positive predictive value. The values were less than 60%. The direct microbiological examination had the better positive predictive values.

The results obtained with the rapid urease test are in contradiction with those described in many studies, although we performed biopsies in the antrum [21] [22]. Redeen *et al.* showed results over 90% for sensitivity, specifity, and predictive values for RUT when biospies were performed in the antrum [22]. The reliability of the kits and the time required to read the results could be critical factors in the perfomance of the rapid urease test. Van Horn *et al.* showed better sensitivity and specificity when the kit was checked 24 hours later [23]. However, the rapid urease test remains the better option for diagnostic testing because of its lower cost and immediate availability of results, which allow rapid treatment of the patient.

The results obtained with the direct microbiological examination with Gram stain open the door to its use in common practice, as Oyedeji *et al.* had mentioned in Nigeria [24]. He had found a higher prevalence in the use direct examination with Gram staining compared to culture and breath test. It could be an alternative to culture or even to histology, whose implementation conditions and the often high costs are a brake.

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

6. Conclusion

The prevalence of *H. pylori* infection depends on the type of diagnostic tests used. The prevalences detected with the urease test and the direct microbiological examination are similar to those reported in the literature for Cameroon. The direct microbiological examination showed good results in terms of sensitivity and specificity, as well as a good predictive value. It could be an alternative to pathological examination, which is more costly. The rapid urease test has lower sensitivity and specificity than those found in the different studies, but it still has a good negative predictive value. It remains a more reliable test than the serological test

Author's Contrbutions

Data collection and analysis: Winnie Bekolo and Ilinga Kelly; Writing and corrections: Winnie Bekolo; Corrections: Sepo Sepo David, Nsenga Njapa Guy Roger Ndjitoyap Antonin, Kowo Mathurin, Eloumou Baganka Servais, Noah Noah Dominique, Ankouane Andoulo Firmin, Njoya Oudou; Sudy design: Eloumou Baganka Servais, Ankouane Andoulo Firmin, Eboumbou Carole.

Conflicts of Interest

The authors state that they have no conflict of interest.

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Abbreviations

H. pylori: *Helicobacter pylori*

RUT: Rapid Urea Test

NPV: Negative Predictive Value

PPV: Positive Predictive Value