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MICROBIOLOGICAL EVIDENCE OF *Helicobacter pylori* FROM PATIENTS SUFFERING FROM GASTRODUODENAL DISEASE. (a)

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Résumé

Notre étude a porté sur la recherche de *H.pylori* à partir de biopsies gastriques chez dix patients atteints de maladies gastroduodénales, et ayant subit une endoscopie digestive haute. Cette bactérie a été mise en évidence par le test rapide à l'uréase, l'examen cytologique, l'examen histologique et la culture. Nous avons pu isoler *H.pylori* de sept patients parmi dix patients infectés par *H.pylori*, signalé par le test de l'uréase et l'histologie. Le processus de leur identification est basé sur les caractères morphologiques et biochimiques. Notre recherche est renforcée par l'antibiogramme pouvant guider vers une antibiothérapie visant à éradiquer *H.pylori*.

Mots clés: *H. pylori , biopsies gastriques, maladies gastroduodénales, antibiogramme, éradication*

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Abstract

Studies of gastric biopsies taken from ten hospitalised patients suffering from gastroduodenal diseases and were subjected to a oesogastroduodenal fibroscopy, with a fast urease test, histological, cytological examination and a microbiological culture make the evidence of *H.pylori*. We had isolated *H.pylori* from seven patients among the ten patients having an infection with *H.pylori* announced by histology and urease test. *H.pylori* identification was based on the morphological and biochemical characters. Our research was reinforced by the realization of an antibiogram which can guide us towards an antibiotherapy for the eradication of *H.pylori* infection.

Keywords: *H.pylori, gastric biopsies, gastroduodenal diseases, antibiogramm, eradication*

ملخص

أدت دراسة عينات معدية منزوعة بالمنظار اللوبي لعشرة مرضى يعانون بالأمراض المعدية-المعوية، باستعمال اختبار اليوريا السريع, الهستولوجيا, السيتولوجيا و اختبار النمو البكتيري إلى إبراز وجود بكتيريا *H. pylori*. النتائج الايجابية لاختبارات ليوريا و الهيستولوجيا مكننا من عزل *H. Pylori* من سبعة(07) مرضى من أصل عشرة (10) يعانون من الإصابات بـ *H. pylori* لتشخيص *H. pylori* اعتمدنا على الدراسة المورفولوجية و البيوكيمائية لهذه البكتيريا. و لقد دعمنا بحثنا هذا بدراسة رد فعل هذه البكتيريا بالنسبة لمجموعة من المضادات الحيوية التي قد يوجهنا لعلاج حيوي للقضاء على *H. pylori* المحدثة للالتهابات.

الكلمات المفتاحية : ***H. pylori*** *، عينات معدية ، أمراض معدية- معوية ، مضادات الحيوية, العلاج.*

Helicobacter pylori is a common bacterium that is present in millions of people world wide [1, 2]. *H.pylori* is a fastidious Gram-negative, curved rod which is associated with active chronic gastris, a gastroduodenal ulcer disease and in the development of a gastric cancer [3,4,5]. It is the principal cause of chronic active (type B) gastritis [6, 7], and a Co-factor of the gastroduodenal ulcer disease [8,9], and it is a risk factor for gastric cancer [10,11,12].

Many works studied the relationship between the antrale infection by *Helicobacter pylori* and its implication in the genesis of the majority of the chronic gastrities and ulcers [13]. The role of *Helicobacter pylori* in the gastric inflammatory diseases is very discussed, and since, a strong correlation was shown between the presence of this bacterium in the stomach and the existence either of a gastrite, or of a peptic ulcer [2,14].

Helicobacter pylori is also associated with gastric tumoral pathology because it was associated with certain gastric carcinomas [15, 16]. This bacterium which is not found in the human stomach would be either responsible, or a Co-factor of gastric inflammatory diseases is in any case an excellent marker as of these diseases [17].

H.pylori infection can be diagnosed by invasive techniques requiring endoscopy and biopsy (histological examination, culture and rapid urease test) and by non-invasive techniques (serology, the C13-urea breath test and the stool antigen test) [14,18,19]. This infection is difficult to cure, requiring combination therapy with antibiotics and a proton pump inhibitor for eradication [20,21,22].

The goal of this study is to establish from a population of patients having undergone a high digestive endoscopy, with gastric biopsies, for varied digestive symptoms. Following a diagnosis which was carried out by the gastro- enterologist at the court of the fibroscopy, one can go in the search of this bacterium by bacteriological and histological examinations.

MATERIAL AND METHODS

Study carried out on the gastric biopsies coming from 10 patients, reached gastroduodenal diseases (chronic gastritis, duodenal ulcer or gastric ulcer) and outwards antibiotics taken or anti-secretoiries.

These patients presented themselves at jeun and undergone an Oesogastroduodenal fibroscopy. The gastric biopsies were taken up on the level of antrum (2 cm of the pylore) with grips biopsies beforehand disinfected and rinsed [23,24,25]. Three antral biopsies intended for histological examination are fixed in formol at 10 % [23,24].Three to four antral biopsies for bacteriological studies are transported in physiological serum maintained at 4°C and are practiced in less than one hour [23, 26].

Urease test

This test is based on the search for ureasic activity of *H.pylori* [27]. The biopsic fragment deposited in a tube containing the urea-indol, incubated at 37°C. The readings of the results were carried out at the end of 20 and 30 min [25, 28,29,30].

Histological examination

This examination allows visualizing spiral bacteria [6,23]. It carries out on histological cuts prepared from the gastric biopsies, coloured with modified Giemsa colouring, and then observed in strong enlarging focus [6,31].

Cytological examination

This examination is based on the setting in evidence of spiral bacteria by a Gram stain of impressed biopsy [6,31]. The smear is prepared by friction of the biopsy mucus on a side blade, coloured with the modified Gram stain (carbofuxine 0,3%), then observed under the optical microscope [32,33].

Culture

The biopsy is crushed in 1 ml of broth heart-brain so as to release the bacteria. Then the totality of the crush was cultured on two Petri dishes respectively containing the chocolate agar (supplemented with 10% human blood) and the medium based on Wilkings Chalgren enriched by human blood (10%) and antibiotics (vancomycin, cefsulodin and cycloheximid).These plates were incubated for 3 to 5 days at 37°C under microaerophilic conditions [23,29,31,32]. The isolated bacteria were identified as *H. pylori* on the basis of morphological colonies, Gram negative staining, positive urease tests, oxidase and catalase activities [34,35].

Antibiogramm

This test allows the study of the *H.pylori* sensitivity to antibiotics by the methods of diffusion agar on the Muller-Hinton medium agar additionned with 10% human blood, inoculated with inoculum 3,0 Mc Farland [36,37]. The incubation carries out at 37°C during 72h in micro aerobic conditions [24,37].The readings of the results were carried out by the measurements of diameters of inhibition zones [23,24].

RESULTS AND DISCUSSION

The demonstration the pathogenic role of *H.pylori* in the gastroduodenal diseases returns on the search for this bacterium which is frequently necessary [38]. In our study, we sought *H.pylori* according to invasive methods requiring the realization of the gastric biopsies [18,25,29,39,40].

The majority of the patients presented a chronic gastritis and a duodenal ulcer. Ten patients having an infection with *H.pylori* documented by the histology, the urease test and sometimes the cytological test were included. We had isolated seven *H.pylori* (Hp 1, Hp2, Hp3, Hp4, Hp5, Hp6, Hp7) from the ten patients infected with *H.pylori* (table 1).

Table 1: Different invasive methods of ***H.pylori*** research

Patients	Urease test	Cytological test	Histological test	Microbiological Culture	Diagnostic
1	+	+	+	+	CG
2	+	-	+	+	CG
3	+	+	+	+	DU
4	+	-	+	+	CG
5	+	+	+	-	GU
6	+	+	+	-	CG
7	+	+	+	-	DU
8	+	+	+	+	DU
9	+	+	+	+	DU CG
10	+	+	+	+	DU

(+) : Positive, **(-)** : Negative, **CG** : Chronic Gastritis, **DU** : Duodenal Ulcer, **GU** : Gastric ulcer

Urease test

The research, directly on the level of the biopsy is a fast and intense ureasic activity, is a major argument in favour of the presence of *H.pylori* [18,30,41,42]. The urease test gives a positive reaction, represented by the turn of indicator of pH into the pink. This indicates the presence of the bacterium [24,31,39]. Urease produced hydrolysis of the urea normally present in stomach from ammonia and carbon dioxide. Ammonia neutralizes the micro environment of the bacterium by protecting it from gastric acidity [23,43].

Histological examination

The microscopic observations of the histological cuts showed the presence of particular forms of *H.pylori* curved or spiral in the crypts and on the surface of the gastric mucous. (Fig.1)The presences of these forms were conformed by other scientific works [18,24,29,31].

The presence of *H.pylori* on the level of gastric epithelium made in evidence the reports of contact between this bacterium and the epithelial gastric cells, site preferentially of *H. pylori* [44].

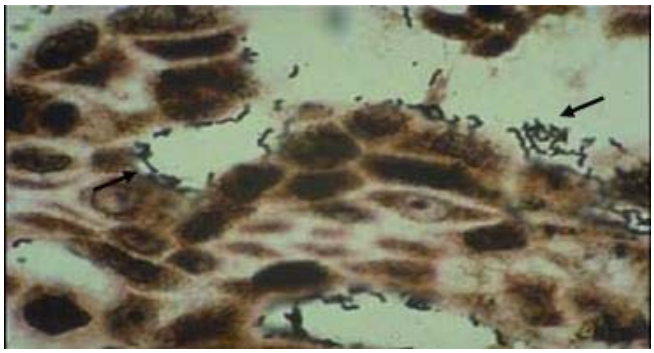

Figure 1: Examination anatomophathology of biopsic gastric by the technique of colouring of "Warthin Starry ", indicating the presence of *H. pylori*

Cytological examination

The cytological examination of gastric biopsy coloured with Gram stain revealed in the majority of the cases the presence of the shapes of curved Gram negative bacilli or slightly **spiral (Fig.2).They** are tendency to be many in certain zones of the smear. The presence of these forms in biopsic imprint was already notified by many authors [18,23,24,29,31]. These authors confirmed the importance of this test which informed us for the presence or the absence of the bacterium.

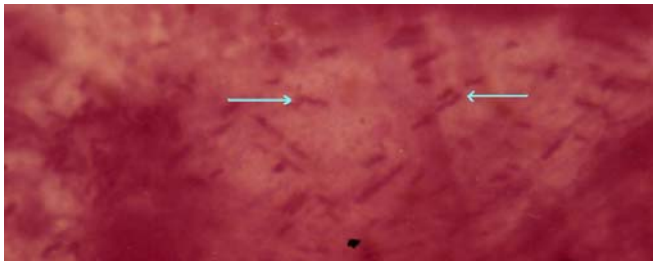

Figure 2: Microscopic observation of a print biopsy after Gram stain

Culture

The morphological methods leave sometimes a doubt contrary to the culture which only allows an unquestionable identification of the bacterium. [38,45]. The process of identification is founded on the morphological and biochemical characters [18,24,29,31].

Macroscopic aspect

The culture of the gastric biopsy on the enriched medium with blood revealed appearance of small colonies, of 1 to 2mm of diameter. They are greys or transparent, gleaming, discreetly convex, round and of regular contour (Fig.3). This aspect corresponded to a *H.pylori* and correlate with different scientists toward the world [6,18,28,29,31,33].

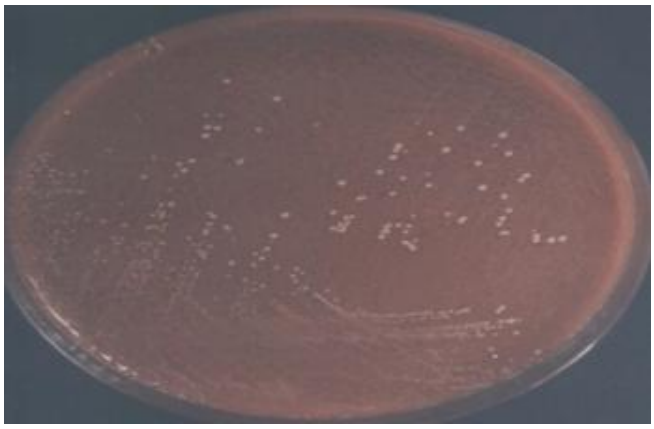

Figure 3: Aspect of the colonies of *H.pylori* developed on chocolate agar after 48h of incubation with 37°C in microaerobiose

Fresh state

The examined bacteria with fresh state appear very mobile by spiral movements and of rotation. This mobility is due a share to its spiral form and different leaves to the presence of polar flagella. [17, 23].

Microscopic aspect

Colonies coloured with Gram stain showed that this bacterium which is negative gram bacilli, curved, in comma or the shape of C, V or S, with regular contour (Fig.4). This morphology is in particular of *H.pylori.* These results correlate with the findings of *H.pylori.* [6, 17, 18, 32].

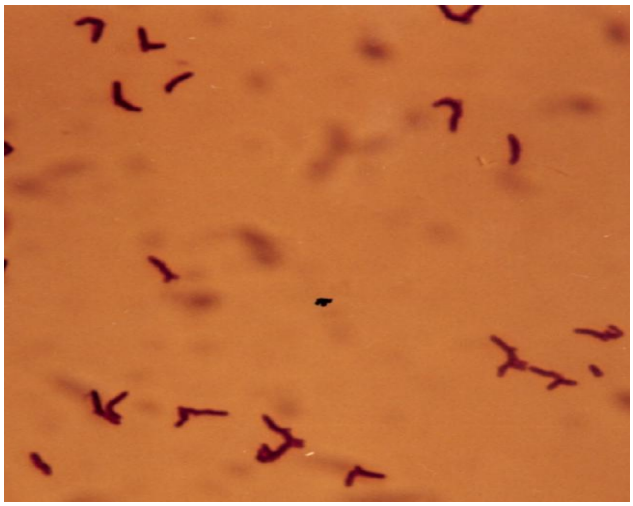

Figure 4: Microscopic observation of ***H.pylori*** after Gram stain from a culture of 48 h on chocolate agar

Biochemical study

Identification is supplemented by the research of the biochemical characters (catalase, oxydase, and urease) [28,29,31]. We noted that the presence of oxydase, catalase and a very powerful urease in all seven isolated bacteria. Based on these biochemical characters we confirmed that was a *H.pylori* [6, 17, 23, 46].

In our study, the microbiological culture was negative among 3 patients from the 10 hospitalised patients having an infection with *H. pylori* announced by the histology, the urease test and sometimes the cytological test. Failure of the culture even in the most tested centers can be explained by the sensitivity of the technique which is extremely depended on the transport conditions, storage and culture provided in the laboratory [25, 29, 46].

In the same way, the irregular distribution of the bacterium in the gastric mucous can contribute to wrongfully negative results [46, 47, 48]. This also explains the existence of the negative results in certain cases of the cytological test (cytology negative whereas infection is present). For that, certain authors recommended the realization of several biopsies at different sites from the gastric mucous, thus improving the output of culture [46,48,31].

Antibiogramm

Study of the *H.pylori* sensitivity to antibiotics, sometimes it is useful in therapeutic choice which showed the excellent activity of the majority of the antibiotics tested.

All strains isolated were sensitive for the majority of antibiotics tested in particular the B-lactamines (amoxicillin, penicillin) where the diameter of inhibition was superior to 21 mm and 29 mm respectively. Also, strains of *H.pylori* present sensitivity against erythromycin, chloramphenicol, tetracycline, gentamycin, metronidazole, tobramycine and furane.

This sensitivity was already signalled by many authors [37, 49, 50, 51]. On the other hand, strains of *H.pylori* showed a remarkable resistance to antibiotics with nalidixic acid, cefsulodin where the diameter of inhibition was inferior to 20 mm and 22 mm respectively, and trimethoprim and sulfamide with the diameters of inhibition were below to 16mm.

This was also quoted by several authors [31, 50, 52]. The resistance of *H.pylori* to antibiotics is one of the major determinants of failure of the treatments for the eradication of the *H.pylori* [5, 49].

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