

## **Comparative estimation of diagnostic tests for helicobacter pylori and the spectrum of gastric mucosal microflora in gastritis and ulcer disease**

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Opening in 1983, Helicobacter pylori (HP) — spiral bacteria colonizing the gastric mucosa (GM), associated with the names of Australian scientists B. Marshall and J. Warren [36]. Over the past 30 years has been established etiological role in the development of HP non-atrophic antral chronic gastritis (CG) type B, which is the name HP-associated CG, as well as in the pathogenesis of HP-associated forms of peptic ulcer (PU), distal gastric cancer (GC) and maltoma (MALT-lymphoma) gastric low-grade [17].

However, there is no doubt that PU and GC are not local infectious pathological processes in the stomach, but the common poli-etiological gastroenterological diseases with a complex pathogenesis, the development of which HP infection plays an important, but not decisive role. This suggests the possibility of developing PU and GC without HP: HP-negativnye form of PU, the frequency of which is 20–30% of duodenal and 40–50% of gastric PU [18, 19, 20, 22, 23, 26, 31, 33], and a proximal (cardial) GC, also not connected with HP-infection [15, 21, 27, 29, 30, 32, 34]. An outstanding clinician and scientist V. X. Vasilenko: [1] states: "PU is a local manifestation of some common disorders". R. Soggea [27] is one of the most respected researchers studying the problem of GC — considers the development of GC as multi-factorial and multi-step process. According to the Sydney classification system, there are HP-independent forms of CG: autoimmune chronic hepatitis (type A), toxic-chemical CG (type C) and special forms of CG (eosinophilic, granulomatous, radiation) [8, 35].

As it is known, HP-infection widespread in the world: 60% of the population in all continents of the earth is infected with HP, however, PU develops only in 12–15% of the infected, distal GC — in 1%, and stomach maltoma— in 0.5% [30, 32]. The majority (70%) of infected people are healthy (asymptomatic) bacteria carriers, often lifelong. However, accurate diagnosis of HP-infection is important, especially when HP-associated with CG, PU and GC, to support eradication therapy [9, 15, 32, 34].

### **Comparative evaluation of various diagnostic tests determining *Helicobacter pylori***

Over the years, developed a number of diagnostic techniques — invasive and non-invasive. Among the invasive methods has been recognized histological method of determining the HP biopsies stained with methylene blue, Gram, Giemsa or Vartin–Starry (sensitivity 90%, specificity 97%). At one time it was even called the gold standard in the diagnosis of HP [9]. Urease rapid test with biopsy material appeared to be especially convenient for practitioners.

For mass epidemiological surveys are most suitable method for determining serum of antibodies — immunoglobulin (Ig) G and A classes — to HP in blood serum (sensitivity 64.0–98.4%, specificity of 88.4–95.0%).

Because of non-invasive diagnostic methods HP received high praise urea <sup>13</sup>C-urea breath test (sensitivity 64–99%, specificity 75–95%); method for determining the HP antigens in faeces (antigen HP determination in feces — NRBA) using the test system (sensitivity 92–94%, specificity 94–97%) and polymerase chain reaction (sensitivity 96.7%, specificity 100.0%) [7, 28, 34]. Importantly, the study of the efficiency of HP eradication should be conducted not earlier than 4 weeks eating after treatment [28].

At the same time have been established and some drawbacks of the tests used for the diagnosis of HP-infection. Thus, when using serological detection of serum antibodies to HP can't exclude the possibility of cross-reaction of antibodies. In addition, antibodies for HP remain in the blood for 6 months after successful eradication of the pathogen, which does not apply this method to evaluate the effectiveness of a course of eradication therapy. When using the polymerase chain

reaction to errors, due to the similarity of the DNA fragments of HP and other microorganisms. Urease tests do not provide reliable results, as the urease activity is not unique to HP, but other mucosal microflora (M-microflora), colonizing GM upon CG, PU and GC. When cytological and histological examination of biopsy samples GM erroneous conclusion is possible because of the proximity of the structure of HP and some other micro-organisms found in the stomach. [7]

Underdiagnosed HP-infection may be due to either a low HP-colonization of GM biopsies or immunodeficiency. [7]

Basic requirements (criteria) to be met by test to determine the presence of the HP in GM:

- high sensitivity and specificity;
- simplicity (accessibility);
- no need for deficit equipment;
- quick response;
- minimum material costs (efficiency).

Maastricht consensus-4 on the diagnosis and treatment of HP-associated diseases (Dublin, 2011), recommends the adoption of a decision on the appointment of antibacterial and anti-secretory agents as diagnostic methods for rapid urease test, a serological test detecting antibodies to HP; urea breath test with <sup>13</sup>C-urea and NREA, giving preference to the latter two.

Some researchers believe that the diagnosis of HP-infection should be comprehensive [6, 28]. However, until now not identified criteria for selecting tests to be included in this set.

Thus, many of the issues associated with the identification of HP in patients with gastroduodenal diseases associated with HP-infection, and to determine the optimal set of diagnostic tests that are designed to help clinicians in the essential task — clarifying the role of this organism in the development of these diseases are highly relevant and still waiting for their decision.

We set a goal of this study to develop a standard set of diagnostic tools to determine the HP-infection in patients with gastroduodenal diseases.

## **Material and methods**

To solve the problems, in the hospital endoscopy department of Perm Clinical Center of FMBA of Russia in 2010–2011, we examined 102 patients with gastroduodenal diseases imposed active complaints of epigastric pain and dyspeptic symptoms and gave informed consent to participate in the study. The diagnosis was made in the course of complex clinical, instrumental and laboratory examination, including morphological confirmation of the diagnosis. In the study group, 11 patients diagnosed with PU of stomach, 6 — duodenal PU (DU), 25 — gastroduodenal erosions, 9 — focal or diffuse atrophic CG, 37 — non-atrophic gastritis of antral (pyloric) department, 9 — scar deformation of the duodenal bulb, 2 — duodenal reflux, 1 — superficial gastroduodenitis, 1 — insufficiency of the lower esophageal sphincter and 1 — Barrett's esophagus. The average age of the subjects was  $54.9 \pm 4.93$  years, among them there were 64 (62.9%) men and 46 (45.1%) women.

The complex research to identify HP included clinical, diagnostic, instrumental, morphological, biochemical, bacteriological and immunological methods.

General clinical examination of patients was accompanied by their questionnaires to study history of the disease. In the course of history have been studied (form No 003/u) and outpatient card (form No 025/u-87).

Biological samples or GM or duodenum were prepared by gastroduodenofiberscopy (GDFS) with biopsy of the affected area of the stomach and duodenum. After treatment, the patient's oral antiseptic using sterile forceps endoscope 3 obtained sample with a specific portion of mucous membrane (depending on the localization of the pathological process), they were placed in 0.3–0.5 ml of buffered saline. Some fragments of the biological tissue after removal fiberoscope forceps dissecting needle and removed without purifying the water, placed in 10% neutral formalin solution for 24 hours for light microscopy. The material was dehydrated, degreased and embedded in paraffin histology in the machine by the usual method. With paraffin blocks sectioned thickness of 5 microns to 10–12 slides.

For staining of histological and cytological micropreparations used standard solutions dyestuffs [25].

Seeding of the biological material was carried out after a thorough mixing and homogenizing the biopsy pieces vortexed (Lachema, Czech Republic) in an improved manner to contact *Helicobacter* 2 blood agar plates (with a highly biological additives BioMerieux, France); while one cup of nutrient medium — by smears, followed by incubation with Anaerobic gasifier package (AnaeroHiGas — Compylo Pack HiMedia (India) or Microaerophil Veston Dickinson) (USA). The second piece of the biopsy in order to enrich thioglycolic placed in a nutrient medium for sterility testing (SCS). The third piece was used for the urease test (test strip BioMerieux) (USA). At the same time to obtain reliable results at the same time we have used a test strip with urea (BioMerieux) (France) together with a liquid medium containing urea [14], or ELISA test (Lachema, Czech Republic).

After approximately 2 days of incubation in the standard mode, microscopic examination was performed SCS preparations Gram-stained (in the absence of visual growth of nutrient agar continued temperature control up to 7 days) and did further seeding on nutrient medium for the primary inoculation. After 5–7 days of incubation of biological samples on Petri dishes we evaluated the nature of growth of the microflora. The identification was carried out by HP and its own modified classical methods [2].

Total antibody IgG, IgA, IgM to the antigen CagA were detected in serum by enzyme immunoassay using the test system Gelika-Best-antibodies (JSC "Vector-Best", Russia) on the unit StatFax-303. The analysis results were evaluated as negative (not containing antibodies to the antigen CagA) as suspect (titer less than 1:5) or weakly positive (titre 1:5) as positive (1:10–1:20) or strongly positive (1:40–1:80). We considered diagnostically significant titer of 1:10 and above.

According to the recommendations of the Maastricht consensus-4 (2011), was used in the non-invasive scatological immunoassay with monoclonal anti-HP antibodies (ImmunoCard STAT HpSA, Germany). Before the study sample of feces pre-diluted in 2-fold. Then 3 drops of the resulting solution was introduced into a test

system and observed as one moves sample violet staining control band; interpretation of the results was carried out no earlier than 10–15 minutes. The result was considered negative if there is only painting the line of control, and positive if staining was two lanes (test and control).

At the initial stage of the investigation of the complex methods for detecting HP was expelled urea breath Helic-test because of its refusal of the majority of patients of the study group: the inconvenience of the procedure is in forced static position of the subject in a continuous exhalation of air in the tube apparatus for 15–20 min. In addition, 25 of the first studies appeared all 25 positive samples, which casts doubt on the reliability of this method.

In developing the standard definition of HP-infection in patients with gastroduodenal pathology were used 9 the most common, according to the literature [6, 28] signs: the presence of inflammatory gastroduodenal diseases and PU of the stomach or duodenum, gastroduodenal erosions (according to GDFS), the presence of levels of total antibodies (IgG, IgA, IgM) to the antigen CagA, these histological and cytological studies, confirming the presence of HP biopsy; the positive results of the urease rapid test; the presence of antigen in stool HP (positive determination result HPSA); the presence of ACS with biopsy specimens GM of curved bacilli morphologically similar to HP.

Of the total sample of patients with gastroduodenal diseases were formed 2 groups. The study group consisted of 40 subjects with positive pathogen seeding of the biopsy that confirmed the existence of reliable HP infection; control group consisted of 62 patients without seeding.

Table 1

**The incidence of HP-infection symptoms in patients with gastroduodenal diseases**

Sign	The main group (n = 40)		The control group (n = 62)		R
	abs.	% ± m	abs.	% ± m	
Inflammatory gastroduodenal diseases	18	45,0 ± 7,9	25	40,3 ± 6,2	> 0.2
PU of stomach and duodenum	9	22,5 ± 6,6	6	9,7 ± 3,8	> 0.05

Erosion of GM or duodenum, according to GDFS	26	65,0 ± 7,5	24	38,1 ± 6,2	<0.05
Presence of antibodies to the antigen Cag A in blood serum	21	52,5 ± 7,9	11	17,7 ± 4,8	<0.01
Histological examination data confirming HP-infection	17	27,4 ± 7,1	6	9,6 ± 3,7	<0.05
Cytology data confirming HP-infection	23	57,5 ± 7,8	13	21,0 ± 5,2	<0.01
Positive results of the urease rapid test with biopsy specimens of GM	34	85,0 ± 5,6	10	16,0 ± 4,7	<0.001
Positive results of the determination of HP antigen in feces	20	50,0 ± 7,9	0	0	<0.001
Presence of curved bacilli morphologically similar to HP in the SCS with biopsy specimens of GM	40	100.0	7	11,1 ± 3,9	<0.001

The groups were matched for age, sex, previous infectious diseases, and to identify gastroduodenal diseases ( $p > 0.2$ ).

The standard definition of HP was conducted by WHO methodology [16] with calculation of sensitivity and specificity of the test (set of characters) which characterizes HP-infection. Statistical analysis of the material was carried out using Microsoft Excel spreadsheets 2013 11.5612. 5606.

### **Results and discussion**

Analysis of the incidence of the three clinical and instrumental signs and six laboratory parameters (total of 9 criteria) describing the HP-infection and included in our ongoing study in patients and control group, revealed significant differences in 7 of them (Table 1).

The exceptions were two features that characterize previously transferred gastroduodenal diseases: CG and gastroduodenitis; PU of stomach and duodenum.

Evaluation of the sensitivity and specificity of the remaining seven criteria showed that the maximum (100%), the sensitivity was only one of them — the presence in the SCS with biopsy specimens GM curved bacilli morphologically similar to HP, and the maximum (100%) specificity — the positive results of the determination of the antigen of HP feces (Table 2).

Table 2

**The sensitivity and specificity of the leading features of HP-infection in patients  
with gastroduodenal diseases**

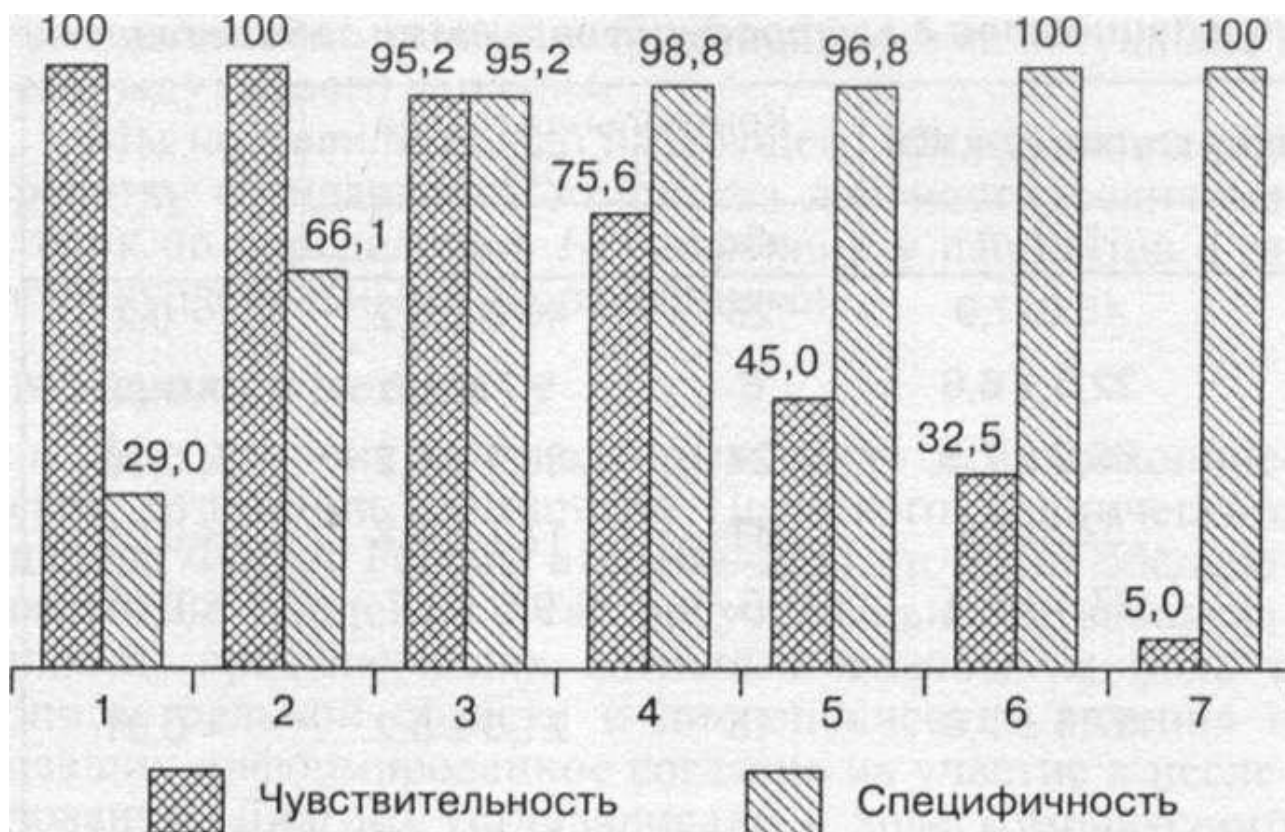
Sign	Sensitivity, %	Specificity, %
Erosion of GM or duodenum, according to GDFS	65.0	61.9
Presence of antibodies to the antigen Cag A in blood serum	52.5	82.3
Histological examination data confirming HP-infection	27.4	90.4
Cytology data confirming HP-infection	57.5	79.0
Positive results of the urease rapid test with biopsy specimens of GM	85.0	84.0
Positive results of the determination of HP antigen in feces	50.0	100
Presence of curved bacilli morphologically similar to HP in the SCS with biopsy specimens of GM	100.0	88.9

The sensitivity of such features as the positive results of the urease rapid test with biopsy specimens of the GM does not exceed 85, 0%. The lowest (27.4%) sensitivity observed by histological method of determining HP. Indicators specificity for most (5 out of 7) features ranged from 61.9 to 90.4%.

Thus, none of the seven listed attributes possessed both high sensitivity and specificity.

Analysis of the frequency of occurrence of combinations of these seven showed signs of high sensitivity, but low specificity (100,0 and 66.1%) with a combination of at least two features (Fig. 1).





**Fig. 1.** Sensitivity and specificity (in %) of combinations of features characterizing HP-infection in patients with gastroduodenal diseases.

In contrast, low sensitivity, but high specificity characterized by a combination of at least four (75.6 and 96.8%), five (45.0 and 96.8%), six (32.5 and 100%) and seven (5.0 and 100%) features. Simultaneously, the high sensitivity and specificity were identified with a combination of at least three features (at 95.2%).

Thus, the diagnosis of HP-infection in patients with gastroduodenal diseases can be installed in the presence of at least three of the following signs:

- definition of HP antigen in feces;
- presence in the blood of total antibodies (IgG, IgA, IgM) to the antigen CagA;
- histological examination of the data, confirming the presence of HP in biopsies;
- cytology data indicating the presence of HP in biopsy;
- positive results of the urease rapid test with biopsy specimens GM;
- presence of curved bacilli morphologically similar to HP in the SCS with biopsy specimens of GM.

The positive results of the urease test, indicating the presence of HP biopsy, were observed in 44 patients with gastroduodenal diseases. Thus 9 (20.5%) patients were isolated from the GM only HP, 25 (56.7%) — HP in combination with other mucosal microflora (M-microflora), and 10 (22.8%) — only M-microflora (excluding HP). Among representatives M-microflora having urease activity, may be mentioned *Staphylococcus aureus*, *St. caprae*, *St. epidermic/is*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Streptococcus salivarius*, *Corynebacterium hoffmanii*, *C. matruchottii*.

Negative results of the urease test have been observed in 52 surveyed, including 6 (10.3%) of them were identified HP at a low concentration (10 CFU/g).

During the study of specificity and sensitivity of the test suite, characterizing the HP-infection was set highly specific feature — positive results HPSA. In view of the foregoing, it is useful to consider this study, the most preferred method for identifying active HP during the clinical examination of patients with gastroduodenal diseases, particularly in high-risk groups, considering the fact that this non-invasive technique which does not require expensive equipment.

Thus, a standard developed by HP to identify patients with gastroduodenal diseases to optimize the diagnosis of the infection in order to reduce the incidence and prevention of clinical complications.

### **The spectrum and frequency allocation mucosal microflora of the gastric mucosa in patients with acute and chronic gastritis and PU disease**

It is known that M-microflora of the stomach, forming microbiocenosis that body may participate in the development of various gastroduodenal diseases, especially chronic hepatitis and PU [26, 27]. Data on the structure of M-microflora of GM in health and gastroduodenal diseases are significantly different [10, 11]. Early efforts studying the microflora of the stomach M-inflammatory and erosive and PU lesions of the stomach and duodenum were few and were carried out on small groups of patients [10, 11, 24].

We studied the species and quantitative composition of the microflora of the M-GM in patients with acute and chronic hepatitis and active PU in the phase of relapse.

On the basis of endoscopic department of Perm Clinical Center of FMBA of Russia we examined 103 patients divided into 2 groups. Group 1 included 61 patients with acute gastritis (AG) or active CG, group 2 — 42 patients with gastric and duodenal PU. The average age of the patients in Group 1 (54.1% men and 45.9% women) was  $46.2 \pm 3.6$  years, group 2 (57.1% men and 42.9% women) —  $52.9 \pm 3.8$  years. The diagnosis was set based on a comprehensive clinical and laboratory examination including histological and cytological examination of GM. When the exhaust gas and CH activity GM biopsy was performed from the area of inflammation at PU — from periulcerous zone.

Biopsy of GM (3 samples) at GDFS was made after pre-treatment the patient's mouth with an antiseptic for the purpose of decontamination accompanying microflora. One sample was used for the manufacture of histological and cytological preparations, the other two were placed in a 0.3–0.5 ml of buffered saline and immediately taken to the bacteriological laboratory. Source culturing a second sample was performed on special nutrient media, including two Petri cups with agar helicobacter bioadditives (Biomeriex). The third sample was placed in a semi-liquid SCS to visualize the growth of microflora, including hardly cultivated form. Bacteriological examination of biopsy GM included qualitative and quantitative determination of aerobic, facultative aerobic, anaerobic microorganisms and fungi genus *Candida*. The primary crop, the cultivation, the study of the morphological, cultural characteristics and identification of isolated microorganisms were performed in accordance with the existing standard documentation and manuals [1, 13, 14]. The studies used standardized and improved culture media, test system express diagnostics firm Lachema and Biomeriex, as well as their own modified method, protected by Russian patents [2, 3, 4, 5]. Digital data is processed using BioStat for Windows (version 4.03) and tables Microsoft Excel.

Analysis of the microflora in patients with GM exhaust and active chronic hepatitis (group 1) revealed 80.3% of the samples in the presence of a variety of microorganisms, including bacteria in the form of associations (55.7%).

Patients PU (group 2) the growth of the microflora was obtained in 90.5% of cases, including in the form of microbial associations (69.4%), which is not significantly different from the rates in group 1 ( $p > 0.2$ ). Total of GM biopsies of patients in group 1 were allocated 105 bacterial isolates, group 2 — 93.

The most common structure of M-microflora of GM in patients with active exhaust and CG (Table 3) met *Streptococcus* spp. (52.5%), ranking the second place was occupied by *Staphylococcus* spp. (23, 0%), the third — the fungi of the genus *Candida* (19.7%). At PU predominant species of microflora were the same *Streptococcus* (57,1%); HP's share was 52.4%, fungi of the genus *Candida* — 40,5%.

In group 1 anaerobic *Peptostreptococcus* spp. was detected in 11.5% of patients, *Enterobacteriaceae* spp. and *Corynebacterium* spp. — in 9.8%. The frequency of detection of other representatives of the microflora (*Neisseria*, *Haemophilus*, *Lactobacillus*, *Bifidobacterium*, *Bacteroides*, *Fusobacterium*, *Veillonella*) was low (less than 6.6% for each), and totaled 24.9%.

Table 3

**The frequency of M-microflora in the GM of patients with AG, CG (group 1) and PU (group 2)**

Microbial Landscape	Group 1 (n = 61)			Group 2 (n = 42)			t	p
	Number of strains		Concentration lgCFU/g	Number of strains		Concentration lgCFU/g		
	abs.	%		abs.	%			
Staphylococcus spp.	14	23.0	2.1	10	23.8	2.2	0.1	> 0.2
Streptococcus spp.	32	52.5	4.4	24	57.1	3.1	0.5	> 0.2
Corynebacterium spp.	6	9.8	3.0	3	7.1	2.3	0.5	> 0.2
Neisseria spp.	4	6.6	3.0	3	7.1	4.3	0.1	> 0.2
Haemophilus spp.	2	3.3	50	1	2.4	5.0	0.3	> 0.2
Enterobacteriaceae spp.	6	9.8	2.8	4	9.5	3.8	0.1	> 0.2
Lactobacillus spp.	2	3.3	3.0	1	2.4	3.0	0.3	> 0.2
Bifidobacterium spp.	2	3.3	20	1	2.4	3.0	0.3	> 0.2
Bacteroides spp.	1	16	3.0	1	2.4	3.0	0.3	> 0.2
Peptostreptococcus spp.	7	11.5	3.0	4	9.5	3.0	0.3	> 0, 2
Fusobacterium spp.	4	6.6	3.0	1	2.4	3.0	eleven	> 0.05
Veillonella spp.	2	3.3	3.0	1	2.4	3.0	0.3	> 0.2
Candida spp.	12	19.7	1.7	17	40.5	1.5	2.3	<0.05
Helicobacter spp.	11	18.0	3.6	22	52.4	3.0	3.8	<0, 001

In group 2, *Peptostreptococcus* spp. and *Enterobacteriaceae* spp. were detected in 9.5%, *Corynebacterium* spp. and *Neisseria* spp. — in 7.1%. Single seeding *Haemophilus* spp., *Lactobacillus* spp., *Bifidobacterium* spp., *Bacteroides* spp., *Fusobacterium* spp. and *Veillonella* in the total population did not exceed 14.3%.

Significant differences in composition of M-microflora in GM patients of the 1st and 2nd groups found between Hp ( $18,0 \pm 4,9\%$  vs.  $52,4 \pm 7,7\%$ ;  $p < 0,001$ ) and fungus *Candida* ( $19,7 \pm 5,1$  vs.  $40,5 \pm 7,6$ ;  $p < 0,05$ ).

In group 1, the highest degree of colonization of GM was marked for *Haemophilus* spp. (5 lgCFU/g), and *Streptococcus* spp. (4.4 lgCFU/g), in group 2 — for *Haemophilus* spp. (5 lgCFU/g) and *Neisseria* spp. (4.3 lgCFU/g). In general, the average concentration of microbial cells in biopsies of GM in group 1 was 3.4 lgCFU/g, in group 2 — 2.7 lgCFU/g, confirming the literature data on the low level of colonization GM by M-microflora [21].

It is important to note that in patients with AG and CG concentration of HP in GM (3.6 lgCFU/g) was inferior only to qualitative indicators colonization of *Haemophilus* spp. (5 lgCFU/g), and *Streptococcus* spp. (4.4 lgCFU/g). In patients with PU at the same level with HP (3 lgCFU/g) was the majority of M-microflora, including normal flora (*Lactobacillus* spp. and *Bifidobacterium* spp.). The lower was just the stage of colonization by *Staphylococcus* spp. (2.2 lgCFU/g), *Corynebacterium* spp. (2.3 lgCFU/g) and fungi of the genus *Candida* (1.5 lgCFU/g). Despite the lower indices of colonization of GM by HP upon PU (3 lgCFU/g against 3.6 lgCFU/g) in this group of patients significantly more frequently met HP-association with other M-microflora (38.1% vs. 13.1%;  $p < 0.01$ ).

The same or similar data were obtained by other authors. So, S. N. Bazlov et al. [12] upon relapse of PU allocated diverse M-microflora from periulcerous area having high enzymatic (including urease) and cytotoxic activity, in an amount of 2.8–5.7 lgCFU/g with a predominance of streptococci (67.7%), staphylococci (62.5%), enterobacteriaceae (46.9), bacteroides (43.7%), fungi of the genus *Candida* (40,6%). At the same time HP were only detected in 34.4% of cases.

Thus, we have found that antral department of a stomach in AG and CG and periulcerous area in PU is colonized, in addition to HP, by other numerous M-microflora having cytotoxic and enzymatic (including urease) activity, which role in the development of these diseases has not yet been investigated and not taken into account.

### **Conclusions**

1. The most informative method of identification HP is a combination of three from among the studied diagnostic features: definition of HP antigen in feces (HPSA); presence of total antibodies in blood serum (immunoglobulins G, A and M) for the antigen CagA; histological (or cytological) study confirming the presence of HP in the biopsy; positive results of the urease rapid biopsy of GM; presence of curved bacilli morphologically similar to HP in the SCS with biopsy specimens of GM.

2. M-microflora of the mucous membrane of the antrum in AG and CG in 52.5% of cases is presented by *Streptococcus* spp., 23% — *Staphylococcus* spp., 19.7% — fungi of the genus *Candida*, 18% — HP.

3. Upon PU in periulcerous area the predominant species of M-microflora are *Streptococcus* spp. (57.1%), HP (52.4%) and fungi of the genus *Candida* (40.5%).

4. Significant differences in the frequency of M-microflora in the stomach in patients with AG, CG and PU were found between HP ( $18,0 \pm 4,9\%$  vs.  $52,4 \pm 7,7\%$ ;  $p < 0,001$ ) and fungi of the genus *Candida* ( $19,7 \pm 5,1\%$  vs.  $40,5 \pm 7,6\%$ ;  $p < 0,05$ ).

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## **Comparative estimation of diagnostic tests for helicobacter pylori and the spectrum of gastric mucosal microflora in gastritis and ulcer disease**

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**Key words:** Helicobacter pylori, informative value of diagnostic tests, species composition of gastric mucosal microflora, gastritis, peptic ulcer

We estimated specificity and sensitivity of diagnostic tests for H. pylori (HP) infection in patients with gastroduodenal problems and studied species composition of gastric mucosal microflora in gastritis and ulcer disease. The following characteristics have been determined as the most informative signs of HP infection: HP fecal antigen, plasma total antibodies (IgG, IgA, IgM) against CagA, histological (cytological) findings confirming the presence of HP antigens in biopsies, rapid urease test, the presence of bent rods morphologically resembling HP in gastric mucosa biopsies cultured in the glycol medium for sterility control. The use of these signs (at least three) in combination ensures efficacious diagnostics of HP infection for the substantiation of its traditional therapy.

The study of the spectrum and occurrence of gastric mucosal microflora revealed the predominance of Streptococcus, Staphylococcus, Candida fungi, and HP in patients with gastritis and Streptococcus, HP and Candida in those with ulcer disease at a mean concentration of microbial cells 3.41 and 2.71 CFU/g respectively. Significant differences were documented only in the occurrence of HP and Candida.