ROLE AND IMPORTANCE OF MICROBIOLOGY TESTING IN THE DIAGNOSIS AND TREATMENT OF HELICOBACTER PYLORI INFECTION

Miglena Stamboliyska[1], Violina Kaludova[2], Milko Mirchev[1], Iskren Kotzev[1], Rosen Madjov[3], Krasimir Metodiev[2]
1) Clinic of Gastroenterology, University Hospital "St. Marina", Medical University - Varna, Bulgaria
2) Department of Preclinical and Clinical sciences, Faculty of Pharmacy, Medical University - Varna, Bulgaria
3) Second Department of Abdominal Surgery, University Hospital "St. Marina", Medical University - Varna, Bulgaria

ABSTRACT

Background: Unsuccessful treatment of Helicobacter pylori (HP) infection is constantly rising. Antibiotic susceptibility testing and selection of individualized therapy are expected to improve eradication of HP.

Aim: To assess the diagnostic accuracy of microbiology testing for HP and to assess the effectiveness of culture-guided susceptibility tested antibiotic therapy.

Materials and methods: A total of 62 HP infected patients, 35 with unsuccessful eradication and 27 treatment naive were subjected to culture testing and 3 endoscopy-based methods. In isolated HP colonies strain susceptibility and resistance were determined by disc-diffusion method. A triple therapy in the naïve group and quadruple in the unsuccessfully treated group was applied. The effectiveness of eradication was evaluated after 2 months by stool antigen test and/or urea breath test. Fisher's exact test for comparison of therapeutic efficacy was used.

Results: HP colonies were isolated in 13 patients - 9 naïve and 4 unsuccessfully treated. Higher sensibility (100%) was shown towards UBT and high specificity between 95% - 100% compared to other methods. Its accuracy was 91.9%. In all patients with proven resistance culture-guided eradication was successful.

Conclusions: Microbiology and antibiotic susceptibility testing allows optimal diagnosis and therapy of HP infection.

Keywords: Helicobacter pylori, eradication, microbiology testing, PCR, antibiotic susceptibility,

Microbiology testing(MT) takes a central place in the so called endoscopy-based strategy for diagnostics of Helicobacter pylori (HP) infection. Along with histology and urea breath test MT is one of the three "golden standard" tests for diagnosis of HP infection.

The rationale for using MT is the possibility for performing antimicrobial susceptibility testing and selecting a proper personalized antibiotic therapy. A variety of randomized controlled trials have proven that HP microbiology followed by antibiotic testing leads to improvement of eradication rates. Culture-guided personalized triple therapy is much more effective than standard triple therapy [1, 2, 3] as a first-line treatment of HP infection.

The aim of the current study is to assess the accuracy of microbiology testing for the diagnosis of HP infection and the effectiveness of culture-guided susceptibility tested antibiotic therapy in a group of Bulgarian patients with HP infection.

PATIENTS AND METHODS:
Patients:

The study included 62HP-infected patients with dyspepsia, of them 34 female and 28male, with mean age - 55±10 years. Of them 35 had unsuccessful eradication and 27 were treatment-naive.

Methods:

All patients had HP infection proven by 3 endoscopy-based invasive methods including tissue sampling: rapid urease test, histology and PCR. Microbiology testing was performed in all cases. When HP colonies were isolated, strain susceptibility and resistance were determined by disc-diffusion method which evaluates areas with growth suppression. As a consequence of the proven antibiotic susceptibility a triple therapy in the naïve group and quadruple in the unsuccessfully treated group was applied. The effectiveness of susceptibility based eradication therapy was evaluated 2 months after initiation by stool antigen test and/or urea breath test.

Statistics: Fisher's exact test was used for assessment of the efficacy of the different treatment regimens. Evaluation of accuracy and precision of culture testing compared to other routine methods was performed, and sensitivity, specificity, positive predictive value (PPD) and negative predictive value (NPD) were calculated. Patients were considered HP positive if they had at least two positive tests for HP and this was accepted as „golden standard". Afterwards the result of each test was separately compared to the „golden standard".

RESULTS:

Baseline characteristics of the patients are shown in Fig.1 and Table 1. All patients had chronic gastritis. The rate

of positivity of the different methods for diagnosing the presence of HP infection is presented in Table 1. HP colonies were isolated in 13 patients–21%, of them 9 naïve and 4 unsuccessfully eradicated.

The result from the culture test coincided completely with histology, the latter being accepted as „golden standard".

Culture vs Histology showed:

Sensitivity – 55 %, specificity – 95.2%, PPV – 84.6% and NPV – 81.6%.

Table 1. Rate of positivity of the different methods for presence of HP infection

	Positivity for HP n (%)	Negativity for HP n (%)
Histology	59 (95)	3 (5)
Rapid urease test	62 (100)	0 (0)
PCR	54 (87)	8 (13)
Urea breath test	43 (69)	19 (31)
Fecal antigen test	55 (88)	7 (12)

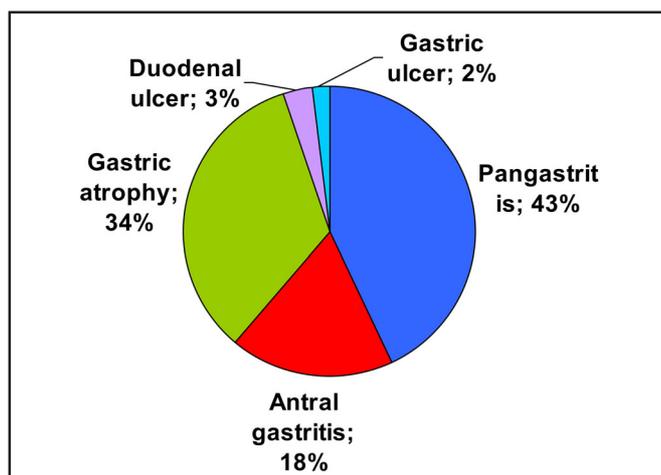**Fig. 1.** Endoscopic diagnosis of the patients with HP infections

Culture vs HP PCR in tissue samples showed:

Sensitivity – 75 %, specificity – 100%, PPV – 100% and NPV – 87.5%.

Culture vs rapid urease test showed:

Sensitivity – 55 %, specificity – 95.2%, PPV – 84.6% and NPV – 81.6%.

Culture vs urea breath test showed:

Sensitivity – 100 %, specificity – 100%, PPV – 100% and NPV – 100%.

Culture vs fecal antigen test showed:

Sensitivity – 31.8 %, specificity – 100%, PPV – 100% and NPV – 50%.

It can be concluded that culture testing has a lower sensitivity compared to other tests which varies between 50% and 100%. The highest sensitivity – 100% is observed when compared to urea breath test and high specificity between 95% - 100% to the remaining methods. There is a full coincidence between culture and urea breath test.

It can be noted from Table 2 that culture test is the most accurate method – 91.9% of the cases are correctly classified. It is followed by histology and rapid urease test which also possess high accuracy close to culture. Fecal antigen test takes the next place.

Table 2. Evaluation of culture and other diagnostic tests

	Sensitivity	Specificity	PPV	NPV	Accuracy
Histology	93.8 %	89.1 %	75 %	97.6 %	90.3 %
Rapid urease test	93.8 %	89.1 %	75 %	97.6 %	90.3 %
Culture test	75 %	89.1 %	92.3 %	91.8 %	**91.9 %**
Fecal Ag test	62.5 %	55.6 %	45.5 %	100 %	58.1 %

Antibiotic susceptibility testing is performed in all 13 patients with isolated HP colonies and acquired strains.

In 9/13 of the cases – 69.23%, of which 5 naïve and 4 treated, antibiotic resistance was found. In 5/13-38.5% the resistance was present to a single agent, in 4/13 – 30.8% - to multiple agents. Clarithromycin- and metronidazole resistance were found in 38.4% of the cases for each antimicrobial agent. Ciprofloxacin resistance was proven in 30.7%

- 4/13 of the cases, amoxicillin resistance - in 1/13 – 7.69%. The same value was present for Levofloxacin. In 8/13 – 61.5% of the patients (5 from the naive and 3 from the treated) data for frequent antibiotic treatment in the past were present.

Resistance in the group of patients with previous unsuccessful eradication (treated)

The group included 3 women and 1 man with mean

age - 52.6 years. Those 4 patients have undergone 8 unsuccessful eradications, 7 of which included standard triple therapy and 1st line sequential eradication.

All 4 patients were found resistant towards different antibiotic agents. Secondary resistance was found in three patients - 75% of the cases towards Clarithromycin and Metronidazole. Ciprofloxacin resistance was proven in 50% of the cases and amoxicillin - in 25%. In two patients resistance towards three and four antibiotics was observed.

The cases with proven resistance and susceptibility testing are as follows:

In Patient No. 1 resistance towards three AB was proven: Clarithromycin, Metronidazole and Ciprofloxacin. Two courses of unsuccessful standard triple therapy were prescribed. Afterwards susceptibility towards amoxicillin, levofloxacin, tetracycline and rifabutin was found and the following quadruple eradication regimen was prescribed:

PPI (40 mg b.i.d.) + Amoxicillin (1000 mg b.i.d.) + tetracycline (500 mg q.i.d.) + Levofloxacin (500 mg b.i.d.).

In patient No. 2 resistance towards four antibiotics was diagnosed: Clarithromycin, Metronidazole, Amoxicillin and Ciprofloxacin. The patient has undergone 3 previous unsuccessful eradications (two courses with standard triple therapy and one sequential). Since susceptibility towards tetracycline, levofloxacin and rifabutin the following quadruple eradication regimen was prescribed:

PPI (40 mg b.i.d.) + tetracycline (500 mg q.i.d.) + Levofloxacin (500 mg b.i.d.) + Rifabutin (150 mg b.i.d.).

In patient No. 3 after two unsuccessful eradications with standard triple therapy, resistance towards clarithromycin was proven. The following quadruple bismuth-containing regimen was prescribed after susceptibility testing:

PPI (40 mg b.i.d.) + De-Nol (240 mg b.i.d.) + Tetracycline (500 mg q.i.d.) + Metronidazole (500 mg b.i.d.).

In patient No. 4 resistance towards metronidazole was discovered after unsuccessful standard triple therapy. Resistance towards amoxicillin, levofloxacin, tetracycline,

rifabutin was found and the following eradication regimen was prescribed:

PPI (40 mg b.i.d.) + Amoxicillin (1000 mg b.i.d.) + Levofloxacin (500 mg b.i.d.).

In patients No. 1, No. 2 and No. 4 frequent use of antibiotics, mainly macrolides, due to respiratory infections, as well as quinolones and other antibiotics for urinary tract and gynecologic infections in adulthood was present. Patient No. 2 reported for frequent use of macrolides and penicillin for acute upper respiratory tract infections in childhood. Patient No. 4 reported for frequent use of metronidazole for gynecologic infections.

In all patient HP infection was successfully eradicated. Follow-up was performed every six months, and one year after treatment no presence of HP was noted.

Resistance and susceptibility in treatment-naïve patients

The group includes 6 female and 3 male patients with mean age 57.8 years. Antibiotic resistance was present in 5 of them – 55.5% (4 female and 1 male).

Among treatment-naïve patients primary resistance towards clarithromycin was found in 2/9 of the cases – 22.2%. The same percentage was present for ciprofloxacin and metronidazole. In one patient levofloxacin resistance was proven. In two patients combined resistance towards ciprofloxacin and metronidazole, and ciprofloxacin and levofloxacin, respectively, was discovered.

Susceptibility towards each antibiotic agent was tested and personalized treatment according to the results was prescribed.

All patients achieved successful eradication with susceptibility-guided therapy. In the remaining subjects no resistance was found and the prescribed susceptibility-guided triple therapy was successful.

Table 3 summarizes the results of all hypotheses testing for the difference of the effectiveness of each therapeutic regimen.

Table 3. Hypothesis testing for the difference of the effectiveness of each therapeutic regimen

	Quadruple concomitant	Quadruple bismuth-containing	1st line sequential	Sequential with probiotic	Salvage	Suseptibility-guided
Standard triple	0,086	0,018*	0,016*	0,005*	0,084	0,001*
Quadruple concomitant		0,823	1	0,687	0,403	0,076
Quadruple bismuth-containing			0,619	1	0,651	0,086
1st line sequential				0,425	0,409	0,033*
Sequential with probiotic					0,661	0,104
Salvage						1

The effectiveness of the standard triple therapy significantly differs from the quadruple eradication regimen: bismuth-containing, 1st line sequential, sequential with probiotic, 2nd line sequential. Susceptibility-guided /triple and quadruple/ therapy is statistically significantly more effective than standard triple and any other empiric therapy.

The standard triple therapy as 1st line is effective in 23 cases – 53.4 % PP and 42 % ITT(95% CI 28 – 55 %), ineffective in 20cases – 46.6%. Side effects were observed in 50% of the cases. The cost of treatment comes up to 137.80 lv.

Susceptibility-guided (triple or quadruple) therapy is effective in12 cases - 100% PP and92% ITT. Side effects were encountered in 30.7%. The cost of treatment varies depending on the preferred antibiotic agent. The total expenses after adding the cost of susceptibility testing varies between 160 and 220 lv.

DISCUSSION:

Culture testing along with histology and urea breath test is considered one of the three most reliable tests for detecting infection with HP – the so called "golden standard" [4]. In terms of all parameters: sensitivity, specificity, positive predictive value, negative predictive value, accuracy, the cited values for culture treatment are 96%, 100%, 100%, 80%, 97%, respectively [5, 6]. Mangusoet al in 2011 [7] believe that culture has the highest accuracy - 92.5% for diagnosis of HP infection compared to 4 other routine tests. According to our results histology and rapid urease test have the highest sensitivity - 93.8%, followed by culture - 75% and stool antigen test - 62.5%.Regarding specificity the value for the three tests is 89%.In keeping with other authors` statements [8] we believe that the lower sensitivity rates of culture are probably due to density of HP bacteria and degree of colonization, as well as presence of gastric atrophy and intestinal metaplasia. These are some of the few inconveniences of the method in particular: capricious, exacting and labor-consuming, requiring professional qualification and competence. Attention should be paid to the preparatory measures before obtaining tissue samples and transport of the latter to the microbiology lab. According to our data culture testing has the accuracy and preciseness - 91.9%, followed by histology and rapid urease test - 90.3%. The lowest accuracy was found for the stool antigen test - 58.1 % which is similar to other authors [5, 7, 9]. These results classify the method among those with high diagnostic yield, especially in cases with frequent recurrent HP infections, where it is able to provide reliable and accurate diagnosis. In cases where presence or absence of HP is debatable, its implementation as a verifying method is highly recommended.

The interest towards culture is mainly due to the possibility for antibiotic susceptibility testing and selection of proper personalized therapy. In recent years the effectiveness of the prescribed eradication regimen is unsatisfactory and unacceptably low. The reason for this is attributed to the high antibiotic resistance towards clarithromycin - the key antimicrobial agent. Nowadays, due to the high clarithromycin resistance in the last 15-20 years, the eradication rate following macrolide therapy has dropped dramatically below 75%.This is the main risk factor for the unsuccessful standard triple therapy including clarithromycin. There are enough data from randomized controlled studies [1, 2, 3, 10, 11] proving that culture followed by antibiotic susceptibility testing result in improvement of the eradication rate. One of the main tasks of the contemporary HP therapy is to minimize the rate of unsuccessful eradication. The analysis of our results underscores the fact that antibiotic resistance strongly correlates with eradication failure. In keeping with data from literature [1, 2, 11] our analysis shows that susceptibility-guided eradication therapy is the most successful one with 100% effectiveness when applied as triple or quadruple. Also, it shows optimal results in terms of compliance, side effects and cost. It should be preferred as initial therapy in cases with frequent antibiotic treatment or suspected antibiotic resistance. Therefore, in regions and populations with high antibiotic resistance (towards clarithromycin, quinolones and metronidazole) culture combined with antibiotic susceptibility testing is promising and should be performed before initial therapy. According to Boyanova et al. [12, 13] in the last 15 years clarithromycin resistance in Bulgaria has raised more than 2 times and is currently above 20%, which necessitates performing culture with antibiotic susceptibility testing in all cases with failed standard triple therapy. Compared to standard triple or any other empiric, susceptibility-guided therapy is not only more effective but also more profitable. Susceptibility-guided eradication is obligatory after two unsuccessful therapies, which is the official recommendation of Maastricht III and IV guidelines [10]. We support the idea that the perfect 100% effective 1st line therapy is antibiotic susceptibility-guided triple therapy and the perfect 2nd- and 3rd line therapy is antibiotic susceptibility-guided quadruple one.

After summarizing and thorough analyzing of our results we propose the following indications for microbiology testing:

- All questionable cases in which the diagnosis of HP is ambiguous.

- All patients previously treated with macrolides or other antibiotics where resistance is suspected.

- All cases with one failed standard triple or other clarithromycin-containing therapy

- All cases with persisting or recurrent HP infection

- All cases where 'lege artis' diagnosis and treatment of Helicobacter pylori is pursued.

In **conclusion:** microbiology with antibiotic-susceptibility testing is a method which combines options for accurate diagnosis and optimal effective therapy of Helicobacter pylori infection. The indisputable advantage of this method is the guaranteed accurate diagnosis and successful individualized antibiotic therapy.

REFERENCES:

1. Giorgio F, Principi M, De Francesco V, Zullo A, Losurdo G, Di Leo A, et al. Primary clarithromycin resistance to Helicobacter pylori: is this the main reason for triple therapy failure? *World J Gastrointest Pathophysiol*. 2013 Aug;4(3): 43-46. [PubMed] [CrossRef]

2. Graham DY, Shiotani A. New concepts of resistance in the treatment of Helicobacter pylori infections. *Nat Clin Pract Gastroenterol Hepatol*. 2008 Jun; 5(6):321-331. [PubMed] [CrossRef]

3. Mégraud F. Bacterial resistance of Helicobacterpylori to antibiotics. *J Gastroenterol Hepatol*. 2012 27(Suppl. 1):S27-S28.

4. Redéen S, Petersson F, Törnkrantz E, Levander H, Mårdh E, Borch K. Reliability of diagnostic tests for Helicobacter pylori infection. *Gastroenterol Res Pract*. 2011; 2011: 940650. [PubMed] [CrossRef]

5. Choi YJ, Kim N, Lim J, Jo SY, Shin CM, Lee HS, et al. Accuracy of diagnostic tests for Helicobacter pylori in patients with peptic ulcer bleeding. *Helicobacter*. 2012 Apr;17(2):77-85. [PubMed] [CrossRef]

6. Frenck RW Jr, Fathy HM, Sherif M, Mohran Z, El Mohammedy H, Francis W et al. Sensitivity and specificity of various tests for the diagnosis of Helicobacter pylori in Egyptian children. *Pediatrics* 2006 Oct; 118(4):e1195-1202. [PubMed] [CrossRef]

7. Manguso F, Riccio E, de Nucci G, Aiezza ML, Amato G, Degl' Innocenti L et al. Helicobacter pylori infection in bleeding peptic ulcer patients after non-steroidal antiinflammatory drug consumption. *World J Gastroenterol*. 2011 17(40): 4509-4516. [PubMed] [CrossRef]

8. Shin CM, Kim N, Lee HS, Lee HE, Lee SH, Park YS, et al. Validation of diagnostic tests for Helicobacter pylori with regard to grade of atrophic gastritis and/or intestinal metaplasia. *Helicobacter*. 2009;14(6):512-519. [PubMed] [CrossRef]

9. Boyanova L. Diagnostic methods for Helicobacter pylori infection. *Suvr med (Sofia)*. 2009; 60(1-2):5-14. [in Bulgarian]

10. Malfertheiner P, Mégraud F, O'Morain C, Atherton J, Axon AT, Bazzoli F, et al. Management of Helicobacter pylori infection-the Maastricht IV/ Florence Consensus Report. *Gut*. 2012 May;61(5):646-664. [PubMed] [CrossRef]

11. Vakil N, Vaira D. Treatment for H. pylori infection: new challenges with antimicrobial resistance. *J. Clin Gastroenterol*.2013; 47(5):383-388. [PubMed] [CrossRef]

12. Boyanova L, Ilieva J, Gergova G, Davidkov L, Spassova Z, Kamburov V, et al. Numerous risk factors for Helicobacterpyloriantibioticresistance revealed by extended anamnesis: a Bulgarian study. *J Med Microbiol*. 2012 Jan;61(Pt 1):85-93. [PubMed] [CrossRef]

13. Boyanova L, Gergova G, Mitov I. Susceptibility of the clinical strains of Helicobacter pylori towards Metronidazole, Tinidazole, Clarithromycin and Azithromycin. *Bulg Med*. 2011; 5(3):35-39. [in Bulgarian]

14. Stamboliyska M. Current clinical, diagnostic and therapeutic aspects of Helicobacter pylori infection, *Summary of thesis,* 2014. [in Bulgarian]

Address for correspondence:
Assoc. prof. Miglena Stamboliyska, MD, PhD
Clinic of Gastroenterology, UMHAT St. Marina
1, Hristo Smirnenski boul., 9010 Varna, Bulgaria
E-mail: m.stamboliyska@abv.bg