



CULTURES OF HELICOBACTER PYLORI FROM STRING TESTS AND GASTRIC BIOPSIES – ANTIBIOTIC SUSCEPTIBILITY TESTS FOR CASES RESISTANT TO TRIPLE THERAPY

Tănăsescu C.¹, Ilie Mădălina², Chifiriuc Mariana Carmen³, Popa Marcela³

¹ Clinical Hospital Colentina, Bucharest, Romania

² Emergency Hospital, Gastroenterology, Bucharest, Romania,

³ University of Bucharest, Faculty of Biology, Microbiology Immunology Department

Abstract. Background: Since its discovery in 1982, *Helicobacter pylori* infection is recognized as a major cause of gastro-intestinal diseases, including chronic gastritis, peptic ulcer disease and gastric malignancy. Its prevalence is connected to socio-economical conditions and most of the individuals are infected during childhood. Regarding *Helicobacter pylori* treatment, a combination of antibiotics must be given but in certain cases repeated courses of different antibiotics are needed to eradicate *H. pylori*. Eradicating *H. pylori* is still a problem because of the rapidly increasing prevalence of multidrug resistant strains worldwide. **Aim:** In this study we want to emphasize the need for culturing *Helicobacter pylori* for certain cases and to show the resistance rate to different antibiotics. The samples for culture were obtained either by gastric biopsy taken by upper GI endoscopy or by enterotest – a string test with a small capsule swallowed and removed after 1 hour. For the positive cultures, an antibiotic susceptibility was performed. Some of the empirically used antibiotics showed surprisingly an increased rate of resistance. **Materials and methods:** We have selected just the patients resistant to the first line therapy (in our case, Clarithromycin 500 mg bid, Amoxicillin, 1g bid and a Pump Proton Inhibitory drug 20 mg bid) dividing them in two groups. For one group gastric biopsy was done and for the other group, enterotest was performed. The samples taken from gastric biopsy and enterotest were put on special media (*pylori* agar) with microaerophilia for cultivation. Positive culture was obtained after an incubation of three to seven days. To this culture an antibiogram by diffusion method was done. **Results:** Positive cultures were obtained in 17 patients (out of 20) from gastric biopsy and in 15 patients (out of 20) from enterotest. Increased resistance in *Helicobacter pylori* strains was observed for Metronidazole and Clarithromycin, commonly used in first line therapy. On the other side, sensitivity was noticed for: Amoxicillin, Ciprofloxacin, Levofloxacin, Furazolidone, Tetracycline. To the patients, a therapy containing an association of two antibiotics from susceptibility testing and a PPI, was given. A good clinical response and a success rate of 93% at one month after finishing the treatment were obtained. The rest of the patients (7%) were retested by rapid urease test or fecal antigen and if they were still positive a second culture was scheduled. **Discussions:** Many treatment regimens have been proposed for *Helicobacter pylori* management. The initial approach should be based on the prevalence of drug resistance-especially to Metronidazole and Clarithromycin. Strict adherence to the treatment should be highly recommended to the patient. Reinfection needs to be avoided by respecting basic hygiene rules. Culture seems the best option for cases resistant to classic therapy. **Conclusions:** Treating *Helicobacter pylori* can be a challenge when this proves to be resistant to several antibiotics. In these cases, culturing it by gastric biopsy or less invasive, by Enterotest and performing antibiogram afterwards seems an appropriate solution. Cultures have to be examined after at least 72 hours of incubation and negative results can only be certified after seven days of incubation. Metronidazole, a very used antibiotic in first line therapy has a very high resistance and it should be avoided especially if the patient has taken this for other infections. **Keywords:** *Helicobacter pylori*, gastric biopsy, enterotest, triple therapy, antibiotic, resistance, susceptibility, culture, urease test, stool antigen

Mădălina Ilie

Aleea Portocalelor 1-3, 60101, Bucharest, Romania

e-mail: drmadalina@gmail.com

Introduction

The discovery of *Helicobacter pylori* bacteria in 1982 represented a revolution point for concepts and management of gastrointestinal diseases. It is now widely accepted that peptic ulcer may have an infectious etiology, the bacteria *Helicobacter pylori* being incriminated, and that it can be treated with antibiotics. Moreover, this bacterium can trigger various gastrointestinal malignancies, the best example being the MALT lymphoma (mucosa associated lymphoid tissue) whose remission depends on eradication of *Helicobacter pylori* [1]. The importance of detecting *Helicobacter pylori* was recognized in 2005 through the Nobel Prize in medicine given to B. Marshall and R. Warren, who were able to isolate and cultivate bacteria, correlating its presence with gastrointestinal disease. The prevalence of *H. pylori* infection is around 50% globally, 80% in most developing countries [2]. In this study we want to show that *Helicobacter pylori* resistant to triple therapy should be cultured, either from gastric biopsies or from enterotest.

Enterotest is a less invasive procedure proposed by Perez-Trallero, which is also called string test [3,4]. A gelatin capsule fixed by a nylon thread is swallowed by the patient (fig 1). After one hour the thread is removed, the capsule being digested. The distal end can be used to detect *H. pylori* through culture but also by the urease test or PCR [5,6]. Sensitivity varies between 60 and 97% [7].



Figure 1. Enterotest (Leodolter and collab., 2005)

Materials and methods

We have studied two groups of patients, the criteria for inclusion being resistance to first-line triple therapy, represented in our case by: clarithromycin 500 mg, 1tb x2/day, amoxicillin 1g, 1tb x2/day and a proton pump inhibitor (PPI) 20 mg x2/day. To check the resistance to treatment, patients were retested with fecal antigen test at 1 month after treatment.

Eradication schemes used empirically included standard triple therapy [8]: PPI in a dose of 20 mg in 12h (omeprazole, pantoprazole, esomeprazole) or lansoprazole 30 mg in 12 h combined with two antibiotics: the most frequently used are clarithromycin 500 mg at 12h and amoxicillin 1g every 12h for 7 days.

The following can also be used:

- metronidazole 500 mg at 12h + amoxicillin 1g at 12h + PPI 20 mg at 12h for 7 days
- metronidazole 500 mg at 12h + clarithromycin 500 mg at 12h + PPI 20 mg at 12h for 7 days.

Ten years ago the eradication rate was over 95% but nowadays it is only 67-79% due to resistance to antibiotics.

Second line regimens include:

- levofloxacin 500 mg every 12 hours combined with
- amoxicillin 1g at 12h
- PPI in a dose of 20 mg 12h - 7days all with good results for eradication.

“Rescue” therapy is with:

- subsalicylate of bismuth 150 mg, 2tb 4 times/day
- tetracycline 500 mg, 1tb x4/day
- metronidazole 250 mg, 1tb x4/day
- IPP – 20 mg x2/day

“Rescue” therapy is very difficult to administer and compliance is very low due to the large number of tablets [8,9].

Recently, sequential therapy may be used which includes [1]: PPI x 2/day + amoxicillin 1g x 2/day for 5 days followed by PPI x 2/day + clarithromycin + metronidazole 500 mg x2/day, eradication with 90% results [7,10]. This will replace the triple therapy in the future as first-line treatment. For the first group of 20 patients an upper gastrointestinal endoscopy was performed and two gastric biopsy samples were taken, the first used for rapid urease test to certify the actual infection and the second used for *H. pylori* cultures.

For the second group of 20 patients, Enterotest was swallowed and removed after an hour, the wire being first placed in saline solution and then in a special culture medium (*pylori* agar) [11, 12].

Patient selection was made based on both clinical and epigastric pain, heartburn, abdominal discomfort, bloating, persistent symptoms after the first treatment cycle (clarithromycin 500 mg, 1tb x2/day, amoxicillin 1 g, and a PPI 1 tablet of 20 mg twice a day) for a week, and the fecal *H. pylori* antigen persistent 1 month after treatment. To the first group of 20 patients we performed upper gastrointestinal endoscopy with the patients’ written consent in advance and coagulation tests to avoid the risk of gastric bleeding. The most frequent endoscopic appearances were:

antral mucosal oedema and redness (erythematous antral gastritis), or vascularization highly visible (atrophic gastritis), but there were also cases of gastric ulcer or duodenal ulcer.

The urease test contains a red indicator called phenol red. Urease hydrolyzes urea to ammonia, which increases the pH of the sample, changing color from yellow (negative) to red [10]. We mention that these components were distributed in Eppendorf tubes produced in our laboratory, obtaining similar results with CLO-test .

Samples were cultivated on Portagerm pylori transport medium.

Portagerm pylori medium is a semisolid medium containing: peptones (from casein and soy), starch, a mixture of antibiotics to limit the proliferation of microorganisms in oropharyngeal microbiota.

The media were incubated in a microaerophilic atmosphere at 37°C. Cultures were examined between 3-7 days of incubation. A culture can only be considered negative at 7 days after incubation.

Growth of small, circular S-type colonies, observed after 3-4 days on selective media with gastric biopsy was an important criterion for identification of *H. pylori*. Hemolytic activity was not observed immediately but after storage for a few days at 4°C.

Microscopic examination showed the bacteria grown morphology often different from the bacteria present in the biopsy sample, coiled bacilli straight or curved (fig. 2).



Figure 2. Microscopic aspect of *H. pylori* on Gram smear

After the growth of *H. pylori* colonies with typical appearance, an antibiogram was performed by diffusion method using the following substances and antibiotics: amoxicillin, clarithromycin, metronidazole, tetracycline, ciprofloxacin, furazolidone, bismuth subsalicylate, esomeprazole, pantoprazole (fig. 3) [13].



Figure 3. Representation of the experimental model of antibiotic susceptibility testing by disk diffusion method

Results

Helicobacter pylori culture was positive in 17 patients (out of 20) from gastric biopsy and in 15 patients (out of 20) from enterotest. The antibiotics to which resistance was most frequently recorded were metronidazole and clarithromycin, while the strains showed sensibility to: amoxicillin, ciprofloxacin, levofloxacin, furazolidone and tetracycline. Resistance to metronidazole and clarithromycin, first-line antibiotics used in treatment, is a problem with HP infection.

Patients who tested positive in cultures followed the second-line treatment with a combination of two antibiotics chosen according to antibiogram results and a PPI, with a good clinical response and a success rate of 93%. This was demonstrated by testing fecal antigen which proved negative 1 month after the end of treatment.

Conclusions

Helicobacter pylori is a public health problem, especially in Eastern European countries where prevalence is very high, same as the number of cases resistant to first-line treatment (clarithromycin 500 mg, 1 tb x2/day, amoxicillin 1 g tb at 12h and a PPI 20 mg x2/day). Unfortunately *Helicobacter pylori* culture cannot be performed from stool and it requires a gastric biopsy or enterotest [14].

Enterotest is an option in upper digestive endoscopy; it is better tolerated by patients and has similar rates of *H. pylori* determination [15, 16, 17].

For cultures obtained by string test, sensitivity was 75% compared with 85% for gastric biopsies.

Before starting treatment a careful patient history should be taken before prescribing antibiotics,

with the focus on avoiding antibiotics that patients have previously used to treat various infections.

Antibiograms have to be performed in all cases where retesting to verify eradication is done, at one month after the treatment by controlling the urease test or fecal antigen. Microaerophilic culture on special media emphasized that *H. pylori* has higher rates of resistance to metronidazole and clarithromycin.

The microscopic examination of cultured bacteria showed different morphological types than those present in the biopsy sample, for example, straight or curved bacilli.

Discussions

Reinfection should be avoided by intensifying hygiene rules; it occurs via the oral cavity (dental plaque, tongue) and via endoscopy [10]. Testing family members for limiting reinfection rate remains to be proved in clinical trials.

Culturing *Helicobacter pylori* is not routinely done because it requires special media for transportation and growing and the costs are high. For certain cases, especially patients resistant to first line therapy, it must be done. The alternative to culturing and antibiogram is taking antibiotics empirically, which may lead to intestinal dysbiosis and treatment failure.

The advantages are clear; the patients receive treatment according to the antibiogram. The results are very good regarding the eradication rate.

Even if culture was tried to be made from stool it was not managed and it can only be done from gastric biopsies and enterotest [17].

Sequential therapy seems a better alternative to the classic therapy but this needs to be verified in large trials. The disadvantage for the patient of this therapy consists in the difficult schedule of administering the tablets.

So the main factors that influence the treatment outcome are: the patient's adherence to the therapy regimen and drug resistant *Helicobacter pylori*, the main factor being drug resistance, according to the current trials.

Checking *H. pylori* eradication should be done for all patients by stool antigen test or by urea breath test one month after finishing the treatment [18].

For all cases resistant to first line therapy, culture should be implemented.

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