

## ERADICATION OF *HELICOBACTER PYLORI*. THE RESPONSE TO TRIPLE STANDARD THERAPY IN A CENTER OF NORTH-EASTERN ROMANIA

ELENA LUMINIȚA POPOVICI<sup>1,2</sup>, CAMELIA COJOCARIU<sup>1,3\*</sup>, LAURA HUIBAN<sup>1,3</sup>, IRINA GIRLEANU<sup>1,3</sup>, MIHAELA DIMACHE<sup>1,3</sup>, MIHAELA MOSCALU<sup>1</sup>, ANCA TRIFAN<sup>1,3</sup>

<sup>1</sup>“Grigore T Popa” University of Medicine and Pharmacy, Iași, Romania

<sup>2</sup>County Clinical Emergency Hospital of Bacău, Romania

<sup>3</sup>Institute of Gastroenterology and Hepatology, “Sf. Spiridon” County Clinical Emergency Hospital, Iași, Romania

\*corresponding author: [cameliacojocariu@yahoo.com](mailto:cameliacojocariu@yahoo.com)

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### Abstract

Nowadays, the prevalence of antibiotic resistance increases worldwide while the efficacy of standard therapy for *H. pylori* infection eradication decreases. Therefore, there is an immense need to investigate the best therapeutic options to avoid resistance and to obtain complete eradication of the bacteria. Considering the few data on clarithromycin (the most used antibiotic in *H. pylori* infections) resistance in Romania, this study evaluates the response to standard *H. pylori* eradication therapy in the north-eastern region of Romania and, through this, an assessment of clarithromycin resistance in this area. One hundred and thirty patients with proven *H. pylori* infection by the stool antigen test were included in the study. All patients received standard eradication therapy: proton pump inhibitor – amoxicillin - clarithromycin according to the Maastricht V consensus. Assessment of *H. pylori* infection eradication was performed using the stool antigen test two months after the therapy ended. The eradication of *H. pylori* infection was found in 90 patients (79.7%). The low eradication rate raises the suspicion of resistance to clarithromycin in the North-Eastern region of Romania.

### Rezumat

Prevalența rezistenței la antibiotic crește la nivel mondial, iar eficacitatea terapiei standard pentru eradicarea infecției cu *H. pylori* scade. Prin urmare, există o nevoie imensă de a investiga cele mai bune opțiuni terapeutice pentru a evita rezistența și pentru a obține eradicarea completă a acesteia. Luând în considerare puținele date privind rezistența la claritromicină (cel mai utilizat antibiotic în infecțiile cu *H. pylori*) în România, această lucrare își propune să evalueze răspunsul la terapia standard de eradicare a *H. pylori* în regiunea de nord-est a României și, prin aceasta, să evalueze indirect rezistența la claritromicină în această zonă. O sută treisprezece pacienți diagnosticați cu infecție cu *H. pylori* prin testul antigenului fecal au fost incluși în studiu. Toți pacienții au primit terapie standard: inhibitor al pompei de protoni – amoxicilină - claritromicină conform consensului Maastricht V. Evaluarea eradicării infecției cu *H. pylori* a fost efectuată folosind testul antigenului fecal la două luni după ce pacienții au terminat terapia cu antibiotice. Eradicarea infecției cu *H. pylori* a fost constatată la 90 de pacienți (79,7%). Acest procent ridică suspiciunea de rezistență la claritromicină în regiunea de nord-est a României.

**Keywords:** *Helicobacter pylori*, standard eradication therapy, antibiotic resistance, clarithromycin

### Introduction

*H. pylori* infection is one of the most widespread infections in the world (30 - 80% of the world population is infected), and it is associated with peptic ulcer disease, chronic gastritis, gastric adenocarcinoma, gastric mucosa-associated lymphoid tissue (MALT) lymphoma.

The epidemiology of *H. pylori* infection sheds light on geographic, ethnic, and racial differences; the prevalence of *H. pylori* infection is influenced by socio-economic status, urbanization, access to water and medical services [22] and it increases with age, being ~ 10% for people under 30 years (including children and adolescents) and equal to the age of 60 years [10, 14]. *H. pylori* is a gram-negative, spiral

(helix-shaped), scarred, and mobile human pathogen discovered in 1982 by Warren and Marshall, which colonizes the gastric mucosa. The transmission of the infection can be achieved by faecal-oral, oral-oral, human-to-human, or infected water consumption. Transmission through human contact is made especially during intrafamilial childhood, through the use of common objects. The use of antibiotics in children for other conditions can lead to eradication of *H. pylori* [24]. Since 1994, the International Agency for Research on Cancer (IARC) and the World Health Organization (WHO) classified *H. pylori* as a type I carcinogen. In the Correa Evolution Model of Gastric Carcinogenesis, the evolution sequence is from mucosal inflammation to atrophic-metaplasia-dysplasia-carcinoma [4, 14].

The diagnosis of *H. pylori* infection is established using invasive and non-invasive methods depending on the availability, cost, clinical manifestations, etc. [15]. Non-invasive tests are urea breath test, serological tests, monoclonal stool antigen test (sensitivity 88 - 89%, 90% specificity). Invasive tests such as urease test (90% sensitivity, 95 - 100% specificity), histological examination, culture and polymerase chain reaction require upper digestive endoscopy and biopsy specimens (two gastric body samples) [12, 20]. The conditions to be followed for proper diagnosis of *H. pylori* infection presume discontinuation of IPP (proton pump inhibitor) two weeks before; antibiotic administration or bismuth products should be interrupted for at least four weeks [4]. Assessment of eradication should be performed at least eight weeks after the end of treatment, and recommended methods are the urea breath test or stool antigen test [12, 14].

The Maastricht V consensus recommends testing and treating *H. pylori* infection in all infected patients, even asymptomatic patients [14]. The standard eradication regimen is the triple therapy regimen that includes a double dose of IPP, amoxicillin 1 g twice a day (b.i.d.), clarithromycin 500 mg b.i.d. or metronidazole 500 mg b.i.d. for 14 days [10, 14].

The widespread use of antibiotics for other infectious diseases has resulted in increasing antibiotic resistance [2, 25]. Worldwide, resistance to clarithromycin is rising: 30% in Italy and Japan, 40% in Turkey, 50% in China [7, 10, 14, 21]. In areas where antibiotic resistance is increased (> 15%) it is essential to adjust the therapy after antibiotic resistance evaluation [2, 25]. The susceptibility test for clarithromycin can be done by standard methods (culture) or molecular analysis directly from a gastric biopsy [21]. The AMPLIDIAG test is a newer test that identifies *H. pylori* infection and clarithromycin resistance directly from stool or gastric biopsy specimens.

Prolonged use (14 days) of high-dose antibiotics may have adverse effects on the microbiota [10, 14]. The association of some probiotics containing *Lactobacillus sp.* or *Bifidobacterium sp.* may prevent the side effects of antibiotic treatment [18], although the Consensus of Toronto found them unnecessary [8]. It is also cited an increase in antibiotic resistance for other microorganisms (*Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli*) and the increased risk of *Clostridium difficile* infection [13, 27]. Bismuth quadruple therapy could be an alternative for treating *H. pylori* infection achieving higher eradication rates of 88%/ 80% or finding new effective solutions (ex: 2-amino-1,3,4-thiadiazoles, plant extracts or natural compounds etc.) [9, 16, 25]. It is necessary to identify a common eradication strategy of *H. pylori* infection worldwide, adapted to geographic conditions.

The current study aimed to evaluate the eradication rate of *H. pylori* infection in a north-eastern centre in Romania.

## Materials and Methods

### Study design

This study was conducted in the gastroenterology department of Bacău County Hospital, Romania, between January 1 and December 31, 2018; it was an open prospective study conducted to evaluate the response to anti-*H. pylori* treatment in *H. pylori*-positive patients.

We enrolled all patients older than 18 years with *H. pylori* infection after obtaining written informed consent. *H. pylori* status was assessed by stool antigen test, performed after discontinuation of any IPP administration two weeks previously and discontinuation of antibiotics and bismuth salts four weeks earlier. Esophagogastroduodenoscopy (EGD) was performed in all patients with alarming signs – anaemia, weight loss, digestive haemorrhage, refractory abdominal pain, gastric resection, etc., suggesting organic lesions.

The main exclusion criteria from the study were age less than 18 years, patients previously treated for *H. pylori* infection, with decompensated diseases (cardiac/pulmonary/renal, etc., all according to specialist examination), severe mental disorders, pregnancy/breast-feeding, malignancy, previous gastric surgery, previous history of allergic reactions to any medication used in this protocol. The demographic information, history of previous upper gastrointestinal bleeding, smoking habits, concomitant medication and alcohol intake was recorded in questionnaires.

The patients *H. pylori*-infected with no exclusion criteria received treatment according to Maastricht V Consensus with double dose IPP Pantoprazole 40 mg twice daily (b.i.d.) + Amoxicillin 1 g b.i.d. + Clarithromycin 500 mg b.i.d. for 14 days. Two months after completing the treatment, the *H. pylori* eradication was assessed using faecal *H. pylori* antigen test.

The protocol was approved by the local research ethics committee and all patients participating in the study signed informed consent to hospital admission according to local regulations.

### Statistical analysis

Statistical data were obtained using the statistical package SPSS.15 (SPSS Inc., Chicago, IL). Numeric variables were analysed with the Mann–Whitney test. Correlations between response and treatment data and variables that could influence it were analysed with chi-square test. The level of significance was set at  $p < 0.05$ .

## Results and Discussion

One hundred and thirty patients admitted in the gastroenterology department of Bacău County Hospital, Romania, between January 1 and December 31, 2018 with a positive stool antigen test for *H. pylori* were included in the study according to the inclusion/exclusion criteria.

The demographic characteristics of our patients are shown in Table 1: age 18 - 82, mean age  $56 \pm 18.36$ , including 41 men (36.28%, age range 19 - 82 years, median  $58 \pm 18.53$ ) and 72 women (63.72%, age range 22 - 81 years, median  $57 \pm 19.69$ ).

Most of our patients were female (63.72% vs. 36.28%) and most of them were from urban areas (69.91% vs. 30.09%).

**Table I**

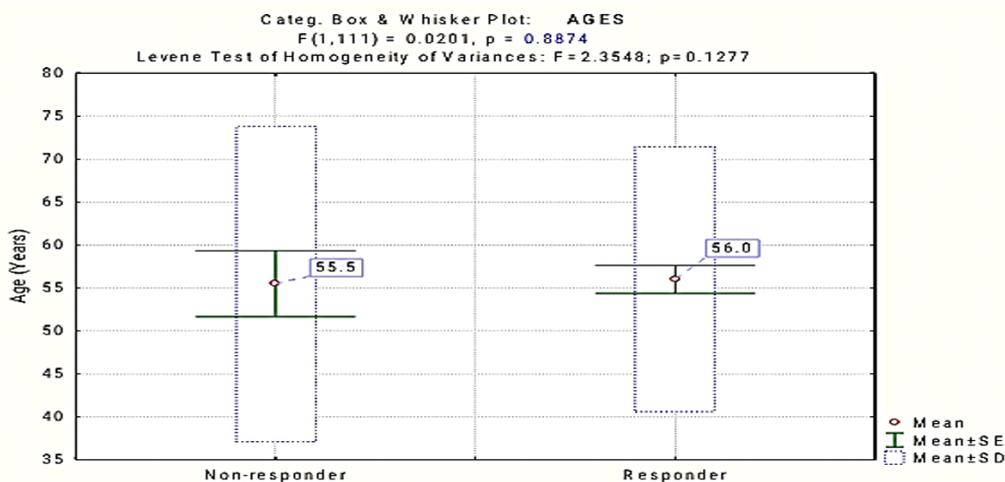
The demographic characteristics of patients

Parameters †	Total	Responsive patients	Nonresponsive patients	p-value
<b>N = 113</b>		<b>90 (79.7%)</b>	<b>23 (20.3%)</b>	
Age: years, mean ± SD		56.01 ± 15.45	55.5 ± 18.36	0.8874
Ages‡				0.9467
< 20	3 (2.7%)	2 (66.7%)	1 (33.3%)	
20 - 40	16 (14.2%)	13 (81.3%)	3 (18.7%)	
40 - 60	43 (38.1%)	35 (81.4%)	8 (18.6%)	
60 - 80	48 (42.5%)	38 (79.2%)	10 (20.8%)	
> 80	3 (2.7%)	2 (66.7%)	1 (33.3%)	
Gender‡				0.3699
Female	72 (63.72%)	55 (76.39%)	17 (23.61%)	
Male	41 (36.28%)	35 (85.37%)	6 (14.63%)	
Environment‡				0.2175
Urban area	79 (69.91%)	60 (75.95%)	19 (24.05%)	
Rural area	34 (30.09%)	30 (88.24%)	4 (11.76%)	
<b>No EGD performed</b>	42	42 (100%)	-	0.0219*
<b>EGD lesions‡</b>	71	48 (67.61%)	23 (32.39%)	
Chronic antral gastritis	27 (38.03%)	15 (55.56%)	12 (44.44%)	
Duodenal ulcer	8 (11.27%)	8 (100%)	-	
Gastric ulcer	10 (14.08%)	9 (90%)	1 (10%)	
Esophagitis	9 (12.68%)	5 (55.55%)	4 (44.44%)	
Erosive gastritis	5 (7.04%)	4 (80%)	1 (20%)	
Atrophic gastritis	2 (2.82%)	1 (50%)	1 (50%)	
Hiatal hernia	10 (14.08%)	6 (60%)	4 (40%)	

† Continuous variables were expressed as mean ± standard deviation; categorical variables: number (%); ‡ Chi-square test (McNemar Chi-square) or Fisher's exact test; (\*) Marked effects are significant at  $p < 0.05$

All 113 *H. pylori*-positive patients included in the study followed the standard eradication therapy of *H. pylori* (double dose IPP Pantoprazole 40 mg b.i.d. + Amoxicillin 1 g b.i.d. + Clarithromycin 500 mg b.i.d.) according to the Maastricht V consensus. No patients discontinued treatment, no significant adverse events

were registered (most adverse events were mild/medium) and the compliance to treatment was excellent – 96.8%. Nevertheless, the eradication rate of *H. pylori* infection was 79.7% – 90 patients and it was higher in males and patients from the urban area.



**Figure 1.**

Age characteristics between groups

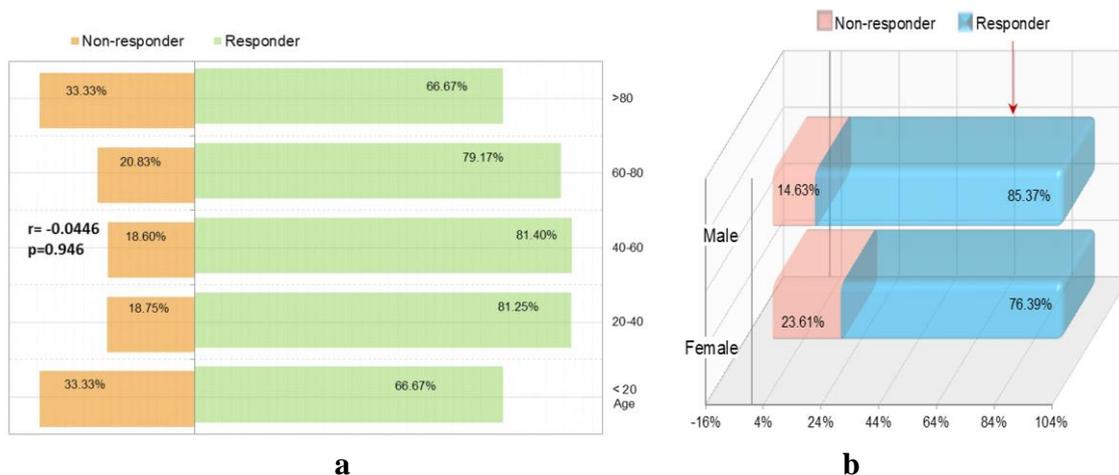
According to demographic characteristics (age, gender, area environment), there were no distinct differences between responders and non-responders (Figures 1, 2 and 3).

There was no significant difference between responders and non-responders in terms of age, the therapeutic success varying between 66.67% and 81.40%; the lowest eradication rates were found in the extreme age groups: under the age of 20 and over 80 years.

A qualitative analysis of the age indicates that the age group 20 - 40 years and 40 - 60 years had a higher

rate of *H. pylori* infection eradication (Figure 2a), but the level of significance (p) and the values of the confidence interval didn't show significant differences ( $p > 0.05$ ).

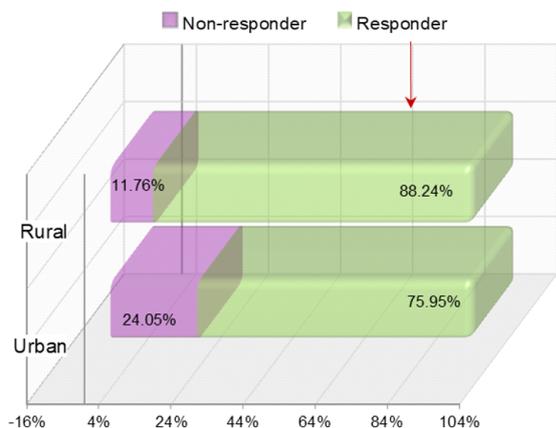
The eradication rate was higher in males (85.37%) (Figure 2b). In these patients, we found more frequently the association between smoking and alcohol consumption. The chance for male patients to respond better to treatment than female patients was 1.803 times higher, but with no statistical significance (95% CI; OR: 0.648 - 5.013,  $p = 0.259$ ).



**Figure 2.** Distribution of responders and non-responders according to age (a) and gender (b)

The female and male frequencies in the two groups are statistically independent (distribution is random) (Test Chi2:  $\chi^2 = 0.235$ ,  $p = 0.628$ ).

The patients in the rural area had a therapeutic response rate above the group average (88.24% vs. 79.7%), but without statistical significance (OR = 2.375, 95% CI: 0.742 - 7.606,  $p = 0.145$ ) (Figure 3).



**Figure 3.** Distribution of responders and non-responders according to patients' area environment

The most common endoscopic finding was antral gastritis (38.03% of patients) and almost half of them

failed to eradicate *H. pylori* infection ( $r = 0.391$ ,  $p = 0.021$ ). The patients with esophagitis, hiatal hernia also rarely responded to treatment but the number of patients is too low for reliable statistical conclusions.

*H. pylori* is the most common chronic bacterial infection in the world, in individuals of all ages and is the principal cause of chronic gastritis, functional dyspepsia, gastric and duodenal ulcer, gastric adenocarcinoma, gastric mucosa-associated lymphoid tissue lymphoma and many extra digestive manifestations (metabolic, haematological, cardiovascular, neurodegenerative, allergic disorders) [28]. The increased prevalence of all these conditions justifies the need to treat *H. pylori* infection to reduce the risk of development of these complications and gastric cancer.

*H. pylori* infection treatment continues to be a challenge for physicians as long as there is no effective treatment that can cure all patients treated. Over the years, many international guidelines for managing patients with *H. pylori* infection have been proposed and periodically updated. The national societies have also updated the guidelines because the rate of eradication of *H. pylori* is affected by several local and regional factors (availability of antimicrobial agents, antibiotic resistance, bacterial peculiarities, etc.) [2, 5].

Our study evaluated the efficacy of the triple combination therapy (IPP Pantoprazole 40 mg b.i.d. + Amoxicillin 1 g b.i.d. + Clarithromycin 500 mg b.i.d.), the standard

regimen for the first-line anti-*H. pylori* treatment. Ilie M *et al.* suggested that Romania is an area of high metronidazole resistance and with low clarithromycin resistance [11].

In our study, the overall eradication rate was 79.7%, lower than expected. The eradication rate in our study is similar only to some extent to that published by Arama S *et al.* The authors reported an 84.2% eradication rate for 14 days of triple therapy and 42.3% for seven days of triple therapy [1]. Simultaneously, however, the rate of eradication in our study is significantly lower than that reported by Preda *et al.* The authors reported an eradication rate of approx. 95% (*per protocol*) and considered that an essential factor of the high therapeutic efficacy was the high dose of IPP (80 mg/day compared with 60 mg/day used by Arama *et al.*) [1, 23]. We used 80 mg IPP daily, and we consider that the early testing of eradication could explain the lower eradication rate in our study compared to data of Preda *et al.* [23].

The *H. pylori* eradication was assessed using faecal *H. pylori* antigen test two months after completing the treatment in our study while Preda *et al.* checked the elimination one month after completing the cure (at this time the false results can be significant).

Many studies have specified that eradication rates of *H. pylori* infection achieved by first-line therapy have decreased to 70 - 85%, due to increasing clarithromycin resistance. The prevalence of primary clarithromycin resistance is greater than 15% in Europe and the eastern Mediterranean region [6] while in the USA, the prevalence of clarithromycin resistance is higher than 20% [25]. These data suggest that the current triple standard therapy for *H. pylori* infection may not be the therapeutic standard worldwide.

The data regarding antibiotic resistance in Romania are limited. A study from the western region of Romania 2006 - 2008 showed high sensitivity (100%) to amoxicillin and erythromycin and high resistance to clarithromycin (33%) and metronidazole (40%), but only ten strains were tested [1]. Ilie *et al.* noted the same resistance for clarithromycin, in contrast, much higher values for resistance to metronidazole (92.8%) and amoxicillin (50%). However, the significance of this study is limited due to the low number of patients included in the study [11].

Otherwise, Preda and Ilie have specified that our country is an area of low clarithromycin resistance and with metronidazole resistance, such that standard triple therapy should be more effective than sequential treatment in Romanian patients [11, 23].

The first clinical study on the antibiotic resistance of *H. pylori* in Romania by determining genetic mutations published by Dumitru *et al.* [6] reported resistance to clarithromycin of 20%. The study included a group of 321 patients from the southern area. Similar to other studies, the most common genetic mutation identified was the A2147G mutation, which is

associated with the highest rate of therapeutic failure of *H. pylori* infection.

In our study, we assessed indirectly the resistance to clarithromycin by determining the response to standard eradication containing clarithromycin regimens. We found similar results (20% response to eradication treatment). However, the two studies were conducted in distinct areas of Romania (the south-eastern region and the north-eastern region) and the study methods were different as well.

The high rate of therapeutic failure (20.3%), compared to the literature data, was unexpected in our study. We don't have a definite explanation for the lack of response to eradication treatment but given the good compliance, we are entitled to think about clarithromycin resistance in this area (the north-eastern region of Romania).

There is a high probability that the high rate of treatment failure and clarithromycin resistance is related to the frequent use of clarithromycin in various infections (urinary, respiratory infections, etc.). Regarding the suggested clarithromycin resistance in our region, we consider that prospective surveillance studies of *H. pylori* antibiotic resistance in Romania must optimize the eradication treatments.

*H. pylori* resistance to clarithromycin has more than doubled in Europe over the last 20 years according to Mégraud *et al.* [17] reaching 21.6% in 2018. The primary resistance to clarithromycin was found to be higher than 15% in Europe in a global meta-analysis spanning 65 countries and 178 studies by Savoldi *et al.* [25]. The success of *H. pylori* eradication regimens based on clarithromycin as a core antibiotic is falling due to increasing resistance to clarithromycin. Unfortunately, no Romanian medical centre participated in any of these studies.

Some studies have noted that *H. pylori* eradication is significantly associated with age. In their research, Mamori *et al.* [16] reported that the only factors that significantly influenced the response to eradication therapy in patients were age (under 50 years) and diarrhoea. The authors consider that the high incidence of adverse events in young patients might explain the low rate of eradication in this group of age. Other studies reported that elderly patients with atrophic gastritis have a higher eradication rate [3]. Our dates do not concur with the results of the study by Mamori *et al.*, but they are somewhat similar to those reported in other Romanian study; in 2016 Arama *et al.* showed that younger patients (aged  $\leq 35$  years) had a significantly better response than older patients [1]. We founded no significant difference between responders and non-responders in terms of age, the rate of eradication varying between 66.67% and 81.40%; the lowest eradication rates were in the extreme age groups: under the age of 20 and over 80 years. The patients between 20 and 60 years old had a success rate of eradication over 81%. The lower rates of elimination

in patients in the extreme age groups may be due to the more frequently reported adverse effects and possibly lower treatment compliance.

Some studies have indicated that female patients have a lower eradication rate explaining this by a possible difference in gastric physiology between males and females, a higher clarithromycin resistance, infection with the *H. pylori* strain with A2143G mutation [19]. However, the cause of gender differences in the eradication rate needs further research. Our results are the same as other studies: the eradication rate was higher in males (85.37%) while in females the eradication was achieved in 76.39% of cases. Our results are similar to those in the literature; we expected a lower eradication rate of *H. pylori* infection in men, considering that we have found that in these patients the alcohol consumption and smoking are risk factors of *H. pylori* failure eradication. It is important to specify that in recent years alcohol consumption and smoking have seen a worrying increase in females. The patients in the rural area had a therapeutic response rate above the group average (88.24% vs. 79.7%), but without statistical significance.

Surprising in terms of the residential environment, different from the results obtained by Arama *et al.*, we found that patients from rural areas had a higher therapeutic response rate than patients of urban regions. It should be emphasized that the group of patients from the rural area was significantly smaller than that of the patients from the metropolitan area, so the results should be interpreted, considering this aspect. The most common endoscopic finding was antral gastritis (38.03% of patients) and almost half of them failed the treatment. We noticed that patients with chronic antral gastritis, atrophic gastritis, esophagitis, and hiatal hernia had a lower rate of eradication, but the number of patients is too small for reliable statistical conclusions. It would have been useful to perform upper digestive endoscopy after the end of the treatment to compare the pre- and post-treatment endoscopic changes in the responders and non-responders.

## Conclusions

In conclusion, *H. pylori* infection treatment continues to be a challenge for physicians. The response to standard eradication therapy in the present study raises suspicion of resistance to clarithromycin in the north-eastern region of Romania. We consider that antibiotic sensitivity testing should be performed in patients in areas with high resistance to clarithromycin, those who have frequently used clarithromycin for various other conditions (respiratory, urinary infections, etc.) and those with the failure of standard eradication therapy for *H. pylori* infection. It is necessary to identify the customized management for eradicating *H. pylori* infection, depending on the resistance to antibiotics and area characteristics.

## Conflict of interest

The authors declare no conflict of interest.

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