The discovery of Helicobacter pylori (H. pylori) provided a possibility to cure effectively patients with peptic ulcers. Recent studies have shown varying susceptibility of H. pylori strains to antibiotics and increasing resistance to some of the recommended drugs. The purpose of the present study was to evaluate the efficacy of the currently recommended eradication schemes and to assess the increasing resistance of H. pylori strains to recommended antibiotics. Furthermore, the effect of probiotics (Lacidofil) on the efficacy of first-line treatment with amoxicillin and clarithromycin was analyzed. The study population consisted of 641 patients: 192 received amoxicillin with clarithromycin and proton pump inhibitor (PPI), 241 - tetracycline, tinidazole, bismuth and PPI, 53 - amoxicillin with clarithromycin and PPI supplemented with Lacidofil. The efficacy of eradication treatment was evaluated by the $^{13}$C-urease breath test. The microbiological examination included 111 samples of H. pylori. The present study demonstrated low efficacy of the tetracycline, tinidazole, bismuth and PPI scheme i.e. 71.4%, moderate efficacy of the amoxicillin with clarithromycin and PPI scheme i.e. 85.9%, whereas the supplementation with Lacidofil significantly increased the efficacy of eradication to 94.3%. The microbiological examination revealed a relatively high level of primary resistance to clarithromycin (22.2%) and a high level to metronidazole (46.7%), with no resistance to amoxicillin. However, the most important finding is the high level of secondary resistance to clarithromycin and metronidazole (more than 66% in both cases). The present findings suggest the need for modification of the recommended eradication schemes.

Key words: Helicobacter pylori, antibiotics, resistance to antibiotics

INTRODUCTION

The discovery of Helicobacter pylori (H. pylori) was a breakthrough in the history of gastroenterology, because it provided a possibility to cure effectively
patients with peptic ulcers. *H. pylori* as a human pathogen is present all over the world. Anti-*H. pylori* antibodies were found in over half (47-75%) of the world population (1, 2). Bielański demonstrated that in the Polish adult population the mean incidence of *H. pylori* infection confirmed by $^{13}$C-urease breath test (UBT) was 69.9% in 1999 (3) and it decreased significantly to 39% within the last 9 years i.e. in the era of mandatory eradication of *H. pylori* in patients with peptic ulcers and gastritis.

Dyspeptic complaints of chronic gastritis occur in about 40% of the infected people, but ulcers develop only in 15-20% (4, 5).

Available evidence shows that the clinical outcome depends not only on the bacterial genotype but also on the immune host response and environmental factors.

A beneficial effect has been achieved in patients with peptic ulceration and mucosa associated lymphoid tissue (MALT) lymphoma. Eradication of *H. pylori* modifies the natural course of peptic ulcer, reducing dramatically relapse rate and permitting permanent cure. After *H. pylori* eradication MALT lymphoma regression was observed in 70-90% of the patients.

Diagnostic tests for *H. pylori* should be performed only in those patients who will receive eradication therapy if infection is detected in compliance with the Maastricht European Consensus guidelines (“test and treat” strategy). Eradication is also recommended in patients with dyspeptic complaints i.e. functional dyspepsia, before initiation of nonsteroidal antiinflammatory treatment and on a case-by-case basis, however, these indications are still controversial.

The main role of *H. pylori* infection in the etiopathogenesis of peptic ulcer and gastritis is now beyond doubt as it has been proved that only bacteria eradication can lead to complete cure of ulcers. The need arises to identify and treat *H. pylori* infection.

As *H. pylori* is a difficult organism to eradicate, resistant to some antibiotics, the indications for treatment and recommended eradication schemes have been established as a result of numerous experimental and multicenter clinical trials.

Recent studies have shown varying sensitivity of *H. pylori* strains to antibiotics in different geographical regions and increasing resistance to some of the recommended drugs. For this reason the current guidelines of the European *Helicobacter Pylori* Study Group (EHPSG) rightly indicate the need to monitor antibiotic resistance and suggest the need to establish regional standards for eradication in the case of increasing resistance to the recommended drugs.

**AIMS OF THE STUDY**

1. To evaluate the efficacy of the currently recommended *H. pylori* eradication schemes;
2. To assess primary and acquired resistance of *H. pylori* strains to metronidazole and clarithromycin;
3. To study the increasing resistance of *H. pylori* strains to amoxicillin;
4. To evaluate the efficacy of first-line treatment with amoxicillin and clarithromycin supplemented with Lacidofil (Institut Rosell Inc., Canada), containing *Lactobacillus acidophilus* and *Lactobacillus rhamnosus*.

**MATERIAL AND METHODS**

The study population consisted of 641 patients receiving treatment in the Clinical Center of Gastrology in Krakow between 1999 and 2002. The study protocol was approved by Local Bioethical Committee of Regional Chamber of Physicians in Cracow and informed written consent was obtained from all randomized patients. There were 256 men and 385 women ranging in age from 18 to 81 years (mean age 44.6 years). Patients were subdivided according to anti-*H. pylori* scheme (Table 1).

**Group IA**

Patients with endoscopically-detected gastric or duodenal ulcers and chronic gastritis with *H. pylori* infection confirmed by UBT, receiving the following treatment: pantoprazole 40 mg +

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Clinical characteristics</th>
<th>Treatment scheme</th>
</tr>
</thead>
<tbody>
<tr>
<td>I A</td>
<td>192</td>
<td>Patients with endoscopically-detected gastric or duodenal ulcers, chronic gastritis and confirmed <em>H. pylori</em> infection (UBT)</td>
<td>PPI (standard dose) + clarithromycin 500 mg + amoxiciline 1000 mg (twice-daily for 10 days)</td>
</tr>
<tr>
<td>I B</td>
<td>241</td>
<td>Patients with endoscopically-detected gastric or duodenal ulcers, chronic gastritis and confirmed <em>H. pylori</em> infection (UBT)</td>
<td>PPI (standard dose) + tetracycline 4 x 500 mg + tinidazole 2 x 500 mg + bismuth salts (Ventrisol) 4 x 120 mg (for 10 days)</td>
</tr>
<tr>
<td>II</td>
<td>45/53</td>
<td>Patients with endoscopically-detected gastric or duodenal ulcers, chronic gastritis and confirmed <em>H. pylori</em> infection (UBT and culture)</td>
<td>45 patients treated according to the antibiogram</td>
</tr>
<tr>
<td>III</td>
<td>66/102</td>
<td>Patients from group I who still had <em>H. pylori</em> infection at 6 weeks after treatment and patients not enrolled in the study or treated unsuccessfully in the past (UBT and culture)</td>
<td>66 patients treated according to the antibiogram</td>
</tr>
<tr>
<td>IV</td>
<td>53</td>
<td>Patients with endoscopically-detected gastric or duodenal ulcers, chronic gastritis and confirmed <em>H. pylori</em> infection (UBT)</td>
<td>PPI (standard dose) + clarithromycin 500 mg + amoxiciline 1000 mg (twice-daily for 10 days), supplemented with Lacidofil containing <em>Lactobacillus acidophilus</em> and <em>Lactobacillus rhamnosus</em></td>
</tr>
</tbody>
</table>
clarithromycin 500 mg + amoxicillin 1000 mg given twice-daily for 10 days in compliance with the current guidelines. The efficacy of eradication was verified using UBT.

**Group IB**

Patients with endoscopically-detected gastric or duodenal ulcers, chronic gastritis and confirmed *H. pylori* infection (by UBT), receiving the following treatment: pantoprazole 40 mg + tetracycline 500 mg + tinidazole 500 mg given 10 days in compliance with the current guidelines. The efficacy of eradication was verified using UBT.

**Group II**

Patients with endoscopically-detected gastric or duodenal ulcers, chronic gastritis, and confirmed (UBT) *H. pylori*, receiving treatment according to the antibiogram against isolated *H. pylori* strain. The efficacy of eradication was verified using UBT.

**Group III**

All patients from group IA and IB who still had *H. pylori* infection at 6 weeks after treatment and patients not enrolled in the study or treated unsuccessfully in the past. In these patients gastroscopy and antibiogram against isolated *H. pylori* strain were repeated. Patients received treatment according to the antibiogram. The efficacy of eradication was verified using UBT.

**Group IV**

Patients as described in group IA receiving the following treatment: pantoprazole, clarithromycin and amoxicilin supplemented with Lacidofil containing *Lactobacillus acidophilus* and *Lactobacillus rhamnosus*.

Prior to supplementation microbiological tests were performed to study *in vitro* the action of various probiotics on the growth of *H. pylori*. Of all probiotics approved for use in Poland, Lacidofil demonstrated the strongest inhibition of *H. pylori* growth in microbiological culture.

The microbiological examination included as many as 111 samples of *H. pylori* strains isolated from gastric mucosa in groups II and III.

• Tissue samples from the prepyloric antrum and gastric mucosa were taken during endoscopy for CLO-test in all patients and in groups II and III additionally for microbiological examination.
• Antral or gastric mucosa samples, after homogenization in a sterile glass jar, were cultured on solid selective medium and supplemented with *Helicobacter agar* (Becton Dickinson, Belgium). Specimens were processed within 2 hours after sampling.
• Samples for bacteriological examination were cultured, gram-stained and tested for urease, oxidase and catalase production. Culture was maintained under microaerophilic conditions in incubators with a brief flow of CO₂ 10%, N₂ 85%, O₂ 5%, at 37°C, for 7 days.
• The susceptibility of isolated *H. pylori* strains to metronidazole, clarithromycin and amoxicilin was assessed quantitatively to establish the lowest concentration of drug that inhibits growth of the infecting organism (minimal inhibitory concentration MIC). The potency of the agent was assessed using the diffusion method with antibiotic dilutions impregnated into gradient absorbent paper strips (E-test, AB – BIODISK, Sweden) in Mueller Hinton Agar with an addition of 7% horse blood (bioMerieux, France). The method allowed for precise determination of MIC in µg/ml as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (74). Resistant strains were defined as those with Etest/MIC results as follows:
\[ \geq 4 \mu g/ml \text{ for amoxicillin,} \\
\geq 1 \mu g/ml \text{ for clarithromycin} \\
>8 \mu g/ml \text{ for metronidazole} \]

Tests were performed in the Microbiological Laboratory, Department of Clinical Physiology of Jagiellonian University Medical College, Cracow, Poland.

- The presence of \textit{H. pylori} infection was verified by the UBT, in which urease produced by \textit{H. pylori} catalyzes the hydrolysis of urea into ammonia and carbon dioxide. Tests were performed in the Isotope Laboratory, Department of Clinical Physiology of Jagiellonian University Medical College (6).

**Statistical analysis**

Treatment results were compared using non-parametric tests: Yates-corrected chi-square test of independence and fraction test. Chi\(^2\) and fraction test were used to compare primary and secondary resistance to antibiotics, and Mann-Whitney test to compare the MICs. A \(p\) value of <0.05 was considered statistically significant.

**RESULTS**

**Clinical characteristics and study results**

We enrolled 641 patients including 256 (39.9\%) males and 385 (60.1\%) females aged from 18 to 81 years (\(x \pm SD: 44 \pm 12.8\)) (Table 2).

Gastroscopy and rapid CLO-test for the presence of urease in gastric samples obtained during gastroscopy were performed in all patients (n=641).

Microbiological tests for the presence of \textit{H. pylori} in gastric biopsy specimens were performed only in groups II and III. Patients received treatment according to the antibiogram against strains isolated from gastric mucosa.

The UBT was performed in all patients who referred for control examination to verify the efficacy of treatment. In group IA of 400 patients treated in the Center of Gastrology 192 patients referred for control examination, in group IB 421 of 640 patients. As one of the aims of the present study was to assess the efficacy of the currently recommended anti-\textit{H. pylori} regimen, only those

<table>
<thead>
<tr>
<th>GROUP</th>
<th>N</th>
<th>Age (lata)</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>X ± SD</td>
<td>Min - max</td>
</tr>
<tr>
<td>IA</td>
<td>192</td>
<td>43,7 ± 10,3</td>
<td>18 – 81</td>
</tr>
<tr>
<td>IB</td>
<td>241</td>
<td>45,1 ± 12,3</td>
<td>20 – 77</td>
</tr>
<tr>
<td>II</td>
<td>53</td>
<td>48,2 ± 14,8</td>
<td>23 – 78</td>
</tr>
<tr>
<td>III</td>
<td>102</td>
<td>43,1 ± 13,4</td>
<td>18 – 81</td>
</tr>
<tr>
<td>IV</td>
<td>53</td>
<td>44,4 ± 13,3</td>
<td>18 – 72</td>
</tr>
<tr>
<td>TOTAL</td>
<td>641</td>
<td>44,6 ± 12,8</td>
<td>18 – 81</td>
</tr>
</tbody>
</table>

Table 2. Characteristics of study groups divided according to age and gender
patients who referred for control UBT were included in analysis. Microbiological tests of gastric biopsy specimens were performed in patients assigned to groups II and III.

**Analysis of eradication efficacy**

All patients with confirmed *H. pylori* infection and indications for eradication received treatment according to the guidelines of the European Helicobacter Pylori Study Group (EHPSG). Two eradication schemes were used as described in Table 1. Patients in groups II and III were treated according to the antibiogram. Patients in group IV received first-line treatment supplemented with Lacidofil. Table 3 summarizes the efficacy of various eradication schemes (in groups IA, IB, II, III and IV).

There was a significant difference in eradication efficacy between group IA and the remaining groups. Most effective was anti-*H. pylori* scheme in group II (treated according to the antibiogram) and group IV (PPI in standard dose + clarithromycin 500 mg + amoxicillin 1000 mg twice-daily for 10 days,

**Table 3. Efficacy of *H. pylori* eradication according to various treatment schemes (groups IA with IB, II, III and IV)**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>n</th>
<th>Repeat UBT</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA</td>
<td>192</td>
<td>165 (85.9%)</td>
<td>27 (14.1%)</td>
</tr>
<tr>
<td>IB</td>
<td>241</td>
<td>172 (71.4%)</td>
<td>69 (28.6%)</td>
</tr>
<tr>
<td>II</td>
<td>53</td>
<td>51 (94.3%)</td>
<td>2 (5.7%)</td>
</tr>
<tr>
<td>III</td>
<td>102</td>
<td>78 (76.5%)</td>
<td>24 (23.5%)</td>
</tr>
<tr>
<td>IV</td>
<td>53</td>
<td>51 (94.3%)</td>
<td>2 (5.7%)</td>
</tr>
</tbody>
</table>

**Table 4. Comparison of susceptibility of *H. pylori* strains to clarithromycin in groups II and III.**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>S</th>
<th>MS</th>
<th>R</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>45</td>
<td>34</td>
<td>75.6</td>
<td>1</td>
<td>2.2</td>
</tr>
<tr>
<td>III</td>
<td>66</td>
<td>21</td>
<td>31.8</td>
<td>1</td>
<td>1.5</td>
</tr>
</tbody>
</table>

S – susceptible; MS – moderately susceptible; R - resistant

**Table 5. Comparison of susceptibility of *H. pylori* strains to metronidazole in groups II and III.**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>S</th>
<th>R</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>45</td>
<td>24</td>
<td>53.3</td>
<td>21</td>
</tr>
<tr>
<td>III</td>
<td>66</td>
<td>22</td>
<td>33.3</td>
<td>44</td>
</tr>
</tbody>
</table>

S – susceptible; R – resistant
supplemented with Lacidofil 2 capsules twice daily for 20 days). The efficacy of eradication in these groups was similar i.e. 94.3%. The same eradication scheme but without Lacidofil (group IA) yielded the efficacy of 85.9%, with the difference between these groups being significant at p=0.04.

The lowest efficacy was observed in group IB (71.4%) receiving tetracycline 500 mg 4 times daily, PPI in standard dose and bismuth salts 120 mg 4 times daily for 10 days. The difference in eradication efficacy between group IA and IB was significant at p<0.001.

Susceptibility of \(H. \text{ pylori}\) strains to antibiotics

Antibiogram was performed against 111 \(H. \text{ pylori}\) strains isolated from gastric mucosa in group II patients who had not been given eradication therapy or any antibiotics for a year (evaluation of primary resistance to antibiotics) and in group III patients in whom anti-\(H. \text{ pylori}\) treatment had been ineffective (evaluation of secondary resistance to antibiotics).

*Figs 2, 3* and *4* show the distribution of \(H. \text{ pylori}\) strains and their susceptibility to amoxicillin, clarithromycin and metronidazole. E-test according to the manufacturer’s interpretation demonstrated that all strains were sensitive to amoxicillin (MIC ≥4 µg/ml for resistant strains) (*Fig. 2*), 54 (48.6%) strains were resistant to clarithromycin (MIC≥1 µg/ml) (*Fig. 3*), and 65 (58.5%) to metronidazole (MIC >8 µg/ml) (*Fig. 4*).

*Fig. 2* shows that the inhibitory concentration of amoxicillin was 0.016 µg/ml for 60 (54%) strains, which means that these strains were susceptible to the lowest concentration of amoxicillin (MIC ranging from 0.016 µg/ml to 256 µg/ml). The MIC for the remaining 40 strains was slightly higher i.e. 0.094 µg/ml, but they were still susceptible to the drug.

*Fig. 3* shows the inhibitory concentrations of clarithromycin (MIC ranging from 0.016 µg/ml to 256 µg/ml). The clarithromycin MIC for 24 resistant strains in both groups was high i.e. 256 µg/ml (only 3 strains in group II and as many as 21 strains in group III).

*Fig. 4* shows the inhibitory concentrations of metronidazole (MIC ranging from 0.016 µg/ml to 256 µg/ml). The metronidazole MIC for 58 (52.2%) resistant strains in both groups was high i.e. 256 µg/ml (37.7% in group II and as many as 62.1% in group III).
Comparing the prevalence of resistance in group II and III, the resistance to clarithromycin was more pronounced in group III i.e. 66.7% than in group II i.e. 22.2% (p<0.001) (Fig. 5).

**Fig. 1.** Comparison of anti-*H. pylori* efficacy according to various treatment schemes between groups IA and IB, and IA and IV.

**Fig. 2.** Activity of amoxicillin against *H. pylori* strains obtained from patients in groups II and III expressed by MIC values (MIC=4µg/ml for resistant strains).

Comparing the prevalence of resistance in group II and III, the resistance to clarithromycin was more pronounced in group III i.e. 66.7% than in group II i.e. 22.2% (p<0.001) (Fig. 5).
**Fig. 3.** Activity of clarithromycin against *H. pylori* strains obtained from patients in groups II and III expressed by MIC values (MIC=1µg/ml for resistant strains).

**Fig. 4.** Activity of metronidazole against *H. pylori* strains obtained from patients in group II and III expressed by MIC values (MIC=1µg/ml for resistant strains).
**Fig. 5.** Comparison of susceptibility of *H. pylori* strains to clarithromycin in groups II and III.

**Fig. 6.** Comparison of susceptibility of *H. pylori* strains to metronidazole in groups II and III.

Fig. 6, comparing susceptibility to metronidazole in groups II and III, shows that resistant strains were more numerous in group III (66.7%) than in group II (46.7%), p<0.005.
The number of strains resistant to clarithromycin and metronidazole was higher in group III (66.7% and 66.7%) than in group II (22.2% and 46.7%). As group III included patients who had been treated with antibiotics it may be concluded that the higher rate of resistant strains in group III was a result of secondary drug resistance. In both groups 33 strains altogether (29.7%) were resistant both to metronidazole and clarithromycin (only 15.5% in group II and 39.3% in group III).

**Fig. 7** compares susceptibility to amoxicillin in groups II and III. In both groups all strains were susceptible but with a visible shift towards higher MIC (0.094 µg/ml) as shown in **Fig. 2**.

**DISCUSSION**

*H. pylori*, considered as the leading cause of gastritis and ulcer, was in 1994 declared by the International Agency for Research on Cancer a type 1 carcinogen (7).

Rising resistance of *H. pylori* strains to the recommended drugs is of major concern. The emergence of resistant strains indicates the need for constant monitoring of *H. pylori* resistance and consequently for the modification of treatment schemes. For this reason the purpose of the present study was to evaluate the efficacy of the currently recommended schemes in *H. pylori*.
eradication, to investigate rising resistance of these \textit{H. pylori} strains in the Polish population, to modify treatment scheme taking into account regional susceptibility to antibiotics and supplementation with probiotics.

The study population consisted of 641 patients including 256 (39.9\%) men and 385 (60.1\%) women ranging in age from 18 to 81 years. Patients were subdivided according to anti-\textit{H. pylori} treatment scheme (groups I A, I B, II, III, IV; Table 2).

According to the study protocol patients were enrolled when gastric biopsy urease test known as CLO-test (\textit{Campylo-like organism-test}) was positive.

Microbiological tests for isolation of \textit{H. pylori} strains and determination of their susceptibility to drugs were performed in group II and III i.e. 155 patients. \textit{H. pylori} was cultured in 111 patients (71.6\%). In group II (patients who had not been treated with antibiotics) \textit{H. pylori} was isolated in 84.9\% of patients, whereas in group III (after unsuccessful eradication treatment) only 64.7\% of patients had a positive microbiological test. Problems in obtaining bacterial cultures in group III may result from much lower colonization after previous antibiotic treatment and the ability of \textit{H. pylori} to produce inactive cocoidal forms. Occupation of the intercellular space and cells by the bacteria poses additional diagnostic difficulties, and, especially, it is impossible to obtain microbiological cultures. False negative results are also encountered in patients receiving drugs reducing the volume of gastric juice such as PPI.

One should take into account the fact that urease and microbiological testing is performed in different biopsy specimens. This may cause discordance of results due to different numbers of viable bacterial cells, which may additionally be at different stages of growth.

All patients with confirmed \textit{H. pylori} infection and indications for eradication received treatment in compliance with the Maastricht European Consensus guidelines 2000 (8). First-line management consisted of a triple-therapy to including amoxicillin, clarithromycin and a PPI. Second-line therapy included metronidazole, tetracycline, bismuth salts and a PPI.

Three different treatment schemes were used and patients were subdivided accordingly to compare eradication efficacy. Patients in groups IA, IB and IV were treated empirically using three different methods. Patients in groups II and III received treatment in accordance with the antibiogram.

Efficacy of anti-\textit{H. pylori} schemes was compared between group IA (PPI, clarithromycin 2x500 mg, amoxicillin 2x1000 mg for 10 days) and IB (PPI, tetracycline 4x500 mg, tinidazole 2x500 mg, bismuth salts 4x120 mg for 10 days) and between group IA versus group II and III (treatment in accordance with antibiogram) and group IV (treatment as in group IA supplemented with Lacidofil).

Patients in group IV received Lacidofil (Rosell Inc., Canada) containing \textit{Lactobacillus acidophilus} and \textit{Lactobacillus rhamnosus}. There are reports (9 - 12) on the antagonistic action of these strains on enteropathogenic bacteria and \textit{H. pylori} increasing the efficacy of eradication. Studies show an inhibiting effect of
Lactobacillus acidophilus LB on urease activity and a strong antagonistic action on H. pylori limiting bacterial growth in vitro, which should also improve eradication success. On the other hand, Lactobacillus has been found to reduce H. pylori-induced gastric inflammation and risk of side effects associated with antibiotic treatment (12-14). In this context supplementation with Lactobacillus strains in accordance with the definition of a priobiotic did not impose additional risk in the present study but it provided a possibility to improve eradication efficacy and to reduce complication rate after antibiotics.

Eradication efficacy was assessed by the $^{13}$C UBT with the reliability being over 93% in previous studies carried out in the Department of Clinical Physiology. The test was performed as recommended not earlier than 6 weeks after antibiotic therapy.

Eradication scheme based on antibiogram (group II) and supplemented with Lacidofil (group IV) was most successful. The eradication rate in these groups was over 94%. In contrast, in group IA (the same drugs but without Lacidofil) the efficacy was 85.9%. The difference between these groups was significant (p=0.04). The lowest efficacy i.e. 71.4% was observed in group IB receiving tetracycline, tinidazole, bismuth salts and a PPI.

In 1994 Bazzoli demonstrated a 100% efficacy of the Maastricht triple therapy (15). The MACH-1 study, a multicenter randomized double-blind study and a meta-analysis by Gisbert et al. in 1999 confirmed the high efficacy of triple therapy with amoxicillin, clarithromycin and a PPI (16). There are also reports on the efficacy of a quadruple therapy including metronidazole, tetracycline, bismuth salts and a PPI. Being better tolerated, this scheme can be used as first-line treatment instead of the triple therapy (17, 18).

These studies show that there are no significant differences in eradication efficacy between triple and quadruple therapy, which disagrees with the present findings. In France, Megraud demonstrated lower efficacy of the recommended schemes and similar differences in eradication rate between triple and quadruple therapy. In the case of eradication failure, he proposed to prolong triple therapy for 2 weeks and to replace clarithromycin with rifampicin. In the case of H. pylori strains resistant to metronidazole in quadruple therapy, he proposed to replace it with furazolidone (19, 20). Megraud recommended that H. pylori resistance to antibiotics should be tested before treatment initiation in areas of lower efficacy of the recommended schemes.

The present findings in group IV are similar to the results of Canducci et al. (10) and Myllyluoma et al. (21) who investigated the effects of first-line triple therapy supplemented with probiotics containing Lactobacillus. Canducci et al. (10) demonstrated low efficacy of standard therapy i.e. 72%, which increased to 88% after the addition of probiotics. Myllyluoma found out that eradication rate increased from 79.2% to 91.3% after supplementation with probiotics. In the present study supplementation with Lactobacillus acidophilus improved eradication efficacy to 94.3%.
Success of anti-\textit{H. pylori} treatment depends on the use of effective drugs, as eradication of bacteria plays a fundamental role in prevention of ulcer relapse. Patient compliance and potential occurrence of side-effects are the additional factors that should be taken into account. Success is more likely in well motivated patients. Each time they should be made aware of the necessity of taking three or more drugs and be informed about their side effects.

The emergence of antibiotic-resistant strains is an important side effect from the microbiological viewpoint. The lower efficacy of triple and quadruple therapy in the present study – similar to France – may result from a very large number of prescriptions for antibiotics in various conditions, thus inducing \textit{H. pylori} strain resistance.

Practically there is no primary strain resistance to amoxicillin. Van Wet \textit{et al.} described \textit{H. pylori} strain resistant to amoxicillin but the patient had been treated unsuccessfully 12 times within 6 years (22). There are reports on a strain isolated in Korea, which was then used in a study on the mechanism of \textit{H. pylori} resistance to amoxicillin (106). In 2001 Głupczyński \textit{et al.} demonstrated that resistance to amoxicillin in Europe is extremely low i.e. 0.8\% (23).

Previously susceptible \textit{H. pylori} strains have acquired resistance to other antibiotics. It is associated with increased use of antibiotics to eradicate \textit{H. pylori} and the widespread use of antimicrobial agents in clinical practice. Clarithromycin is more frequently used for other indications in other countries such as in France, Spain, Italy or Belgium. In most countries primary resistance to clarithromycin is relatively low, whereas secondary i.e. acquired resistance may reach even 52\%.

The rate of resistance to metronidazole is higher in women than in men, probably due to its frequent use to treat gynecological infections (24, 25).

Varying sensitivity of \textit{H. pylori} strains to antibiotics is a fact. For this reason the current guidelines of the EHPSG indicate the need to monitor antibiotic resistance and suggest the need to establish regional standards for eradication in the case of increasing resistance to the recommended drugs. One of the purposes of the present study was to evaluate susceptibility of \textit{H. pylori} strains to the currently recommended antibiotics. E-test MIC antibiogram was performed for 111 \textit{H. pylori} strains isolated in antral gastric biopsy specimens obtained from dyspeptic patients (groups II and III).

In the present study all \textit{H. pylori} strains were susceptible to amoxicillin, confirming the reported lack of resistance to this agent. For 60 (54\%) strains, the inhibitory concentration of amoxicillin was 0.016 µg/ml, which means that these strains were truly susceptible to the lowest concentration of amoxicillin. However, for the 51 strains the MIC value was higher (0.094) but still within the limits of susceptibility. This means that the inhibitory concentration is shifted towards higher values and suggests that strains resistance also to amoxicillin may appear in Poland. Of all strains, 58.5\% were resistant to metronidazole, with the highest MIC values (256 µg/ml) in 52.2\%. It indicates that these strains are highly resistant to...
metronidazole. Eradication efficacy in infections with high-level metronidazole-resistant strains (MIC >32 µg/ml), isolated from patients before and after treatment may be very low. The number of resistant strains was significantly higher in group III (66.7%) than in group II (46.7%), p<0.005. The higher rate of resistant strains in group III may result from secondary resistance, as this group included patients in whom previous eradication scheme had failed. Many investigators emphasize the importance of primary resistance to metronidazole and difficulties in treating *H. pylori* infection in such patients (26 – 29). National and international reports show that the prevalence of strains with primary resistance to metronidazole ranges from 7% to 90% (26, 30, 31). The prevalence of strains with acquired resistance is even higher and ranges from 50% to 90%, which is in agreement with our present findings.

This leads to the conclusion that the currently recommended empirical treatment with metronidazole in Poland with the high prevalence of primary and acquired resistant strains may be ineffective. The high resistance to metronidazole in the present study accounts for the low eradication efficacy in group IB (tinidazole, tetracycline, bismuth salts and PPI). For this reason it is recommended to perform microbiological tests and study susceptibility of *H. pylori* strains in individual patients before administration of metronidazole.

In the present study of 111 strains, 54 (46.6%) were resistant to clarithromycin. Comparing the prevalence of resistance in groups II and III to clarithromycin, similar to metronidazole, was more prevalent in group III (66.7) than in group II (22.2%). Such a big discrepancy indicates that secondary resistance to clarithromycin displays rising tendency.

The essential risk factor for clarithromycin resistance is its widespread use both for eradication of *H. pylori* and for respiratory tract infections, especially that previous consumption of macrolides (erythromycin and azithromycin) almost always induced cross resistance to clarithromycin. In France, where macrolides had been widely used for a long time, the initial rate of *H. pylori* strains resistant to clarithromycin was about 10% (19, 20, 30), but increased to 29.1% in children as shown by Alarcon *et al.* (32). In 2001 in Poland, Rozynek *et al.* documented a lower rate of primary resistance to clarithromycin, i.e. 23.5% (33). Other investigators report a relatively high rate, even 90% (34), which is in agreement with the present findings. Evidence shows that the number of strains resistant to clarithromycin and metronidazole gradually increases.

The present study also addressed double resistance i.e. to metronidazole and clarithromycin. Double resistant strains were detected in 29.5% of patients in group II and III (15.5% in group II and 39.3% in group III). According to French investigators, the rate of double resistant strains was much lower i.e. only about 9% (35).

Most reports on *H. pylori* susceptibility to antibiotics emphasize that resistance has been rising in recent years to cause even greater problems in the future.
The most important finding of the present study is rising resistance of *H. pylori* strains to metronidazole and clarithromycin, which indicates that empirical treatment with these two antibiotics should be abandoned as it does not guarantee eradication efficacy. Furthermore, it places the patient at higher risk for complications such as post-antibiotic (pseudomembranous) colitis or candidiasis due to superinfection with yeast-like fungi.

The present findings suggest the need for modification of the recommended eradication schemes. Clarithromycin and metronidazole should not be used simultaneously (the combination recommended as first-line treatment in the Maastricht consensus) and be limited only to patients with known sensitivity to macrolides and nitroimidazole. The high level of secondary resistance to clarithromycin and metronidazole virtually excludes a possibility of their use as second-line treatment without prior antibiogram.

The present findings and literature data lead to the conclusion that it is justified to define the sensitivity of *H. pylori* strains to drugs, especially in patients after unsuccessful first-line treatment in order to increase therapeutic efficacy and to reduce drug resistance.

The current guidelines of the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) recommend that antibiogram and *H. pylori* resistance to clarithromycin and metronidazole should be determined each time prior to treatment.

Resistance to tetracycline is absent in most countries and for this reason the drug should be prescribed more often. It is also reasonable to consider other, less frequently used antibiotics with preserved susceptibility. Recently, high efficacy of rifabutine, spiramycine, furazolidone and new quinolones: levofloxacine and moxifloxacine has been reported (36 - 39). Further multi-center randomized clinical studies are needed to provide evidence that eradication with a different antibiotic is warranted.

The limitations of the recommended eradication scheme should prompt a search for new modalities in the treatment of *H. pylori* infections with probiotics appearing to be most promising. Supplementation with probiotics:

1. reduces the rate and intensity of side effects, especially post-antibiotic diarrhea
2. prevents selection of resistant strains i.e. *Clostridium* or superinfection with yeast-like fungi i.e. *Candida*
3. assures patient compliance and adherence to the prescribed regimen
4. may improve eradication efficacy via direct antagonistic action of *Lactobacillus* strains on *H. pylori*

An important practical finding in the present study is that Lacidofil increases efficacy of first-line treatment. The addition of *Lactobacillus* strains to eradication therapy does not place the patient at increased risk for unwanted effects but instead it improves eradication efficacy and reduces complications after antibiotic therapy.
However, efficacy of eradication supplemented with probiotics should be confirmed in randomized double-blind placebo-controlled studies.

CONCLUSIONS

1. The most effective scheme in the treatment of *H. pylori* infection is a 10-day triple therapy with a proton pump inhibitor (PPI), clarithromycin 500 mg bid and amoxicillin 1000 mg bid supplemented with Lacidofil containing probiotics *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* strains.
2. The lowest eradication efficacy i.e. 71.4% was observed when using a 10-day quadruple therapy with tinidazole 500 mg bid, tetracycline 500 mg qid, bismuth salts 120 mg qid and a PPI.
3. Patients in whom anti-*H. pylori* treatment had been ineffective has a higher level of resistance to metronidazole and clarithromycin than untreated patients. It indicates rising secondary resistance which may reduce eradication efficacy in the future.
4. The high rate of clarithromycin resistant strains indicates rising *H. pylori* resistance to this antibiotic in the country.
5. The high level of primary resistance to metronidazole and clarithromycin is a contraindication for the use of these two antibiotics as first-line treatment.
6. The very high level of secondary resistance to clarithromycin excludes a possibility of its use as second-line treatment.
7. No amoxicillin resistant strains were isolated but the inhibitory concentration (MIC) was shifted towards higher values, which may predict the appearance of strains resistant to this antibiotic. It is recommended to continue the monitoring of *H. pylori* resistance to amoxicillin.
8. The addition of Lacidofil to eradication therapy improves the treatment efficacy. However, further studies on efficacy of eradication supplemented with probiotics are warranted.

REFERENCES


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