

## PHARMACOLOGY

LEVOFLOXACIN RESISTANCE OF *HELICOBACTER PYLORI* STRAINS ISOLATED FROM PATIENTS IN SOUTHERN POLAND, BETWEEN 2006-2012ELŻBIETA KARCZEWSKA<sup>1\*</sup>, KAROLINA KLESIEWICZ<sup>1</sup>, IZABELA WOJTAS-BONIOR<sup>1</sup>, IWONA SKIBA<sup>1</sup>, EDWARD SITO<sup>2</sup>, KRZYSZTOF CZAJECKI<sup>2</sup>, MAŁGORZATA ZWOLIŃSKA-WCISŁO<sup>3</sup> and ALICJA BUDAK<sup>1</sup><sup>1</sup> Department of Pharmaceutical Microbiology of the Jagiellonian University Medical College, Medyczna 9, 30-688 Kraków, Poland<sup>2</sup> Falck Medycyna Outpatient Clinic of Gastroenterology, Mazowiecka 4–6, 30-036 Kraków, Poland<sup>3</sup> Chair of Gastroenterology, Hepatology and Infectious Diseases, Jagiellonian University Medical College, Śniadeckich 5, 31-531 Kraków, Poland

**Abstract:** An increasing resistance of *Helicobacter pylori* (*H. pylori*) to antimicrobial agents leads to the need of regional monitoring of the prevalence resistant strains (according to the Maastricht/Florence consensus report, 2012). The aim of the study was to assess the resistance to levofloxacin of *H. pylori* strains isolated from adult patients of Małopolska region in Poland. Biopsies taken from gastric mucosa during gastroscopy constituted the material for the study. Two hundred ten *H. pylori* strains were isolated from 811 patients. A majority of strains (171) came from patients before the treatment of *H. pylori* infections while the remaining 39 strains were isolated from patients after the failed therapy. Susceptibility of *H. pylori* to levofloxacin was determined by strips impregnated with antibiotic gradient (E-test, bioMérieux). The obtained minimum inhibitory concentration (MIC) values ranged from 0.002 mg/L to 32 mg/L. The percentage of strains resistant to levofloxacin amounted to 8.10% (17/210). Among the group of strains isolated from patients before the treatment, 5.85% (10/171) of *H. pylori* strains were resistant to levofloxacin. In the group of strains isolated from patients after the treatment 17.95% (7/39) of strains were resistant. The difference in the frequency of *H. pylori* strains resistant to levofloxacin in patients before and after the treatment of the infection due to *H. pylori* was statistically significant ( $p = 0.0297$ ). The low percentage of *H. pylori* strains resistant to levofloxacin justify that the introduction of a triple therapy with levofloxacin is a good alternative in the treatment of *H. pylori* infections, especially in regions with high prevalence of *H. pylori* strains resistant to clarithromycin (> 20%).

**Keywords:** *Helicobacter pylori*, drug-resistance, levofloxacin

*Helicobacter pylori* (*H. pylori*) is one of the most common human pathogens that affected about 50% of the human population (1). *H. pylori* is a Gram-negative, microaerophilic, spiral bacterium that permanently or temporarily inhabits various parts of the gastric mucosa. *H. pylori* is the main pathogenic factor of gastroduodenal diseases: gastritis B, gastric ulcer, duodenal ulcer, gastric cancer, gastric lymphoma MALT (Mucosa Associated Lymphoid Tissue) and Ménétrier's disease. Moreover, various studies reveal a relation of *H. pylori* to the development of coronary heart disease, stomatitis, liver diseases (hepatic encephalopathy, neoplasms), skin diseases and allergy (2–12).

Eradication of *H. pylori* is recommended at every stage of the disease (3). Introduction of the effective therapy guarantees eradication of the infection, which results in the relief and often in the regression of disorder changes that otherwise might lead to gastric cancer, which develops in 2% of individuals infected with *H. pylori* (5, 13, 14).

Indications for the treatment and guidelines for clinical trials are elaborated by the European Helicobacter Study Group (EHSG) (1, 15). On the basis of these recommendations, the Working Group of the Polish Society of Gastroenterology (PTG – pl. Polskie Towarzystwo Gastroenterologiczne) has issued guidelines that are obligatory in Poland.

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Current obligatory recommendations in Poland were established by PTG in 2008 (5) on the basis of Maastricht Consensus 2005 by EHSG. Now, there are no new recommendations based on the newest Maastricht/Florence Consensus 2012. According to all these recommendations, the treatment of *H. pylori* infection is a combined empirical therapy including three types of drugs: gastric antisecretory drugs, cytoprotective drugs and antimicrobial agents.

Current regimens of the treatment of *H. pylori* infections in Poland (2008) are shown in Table 1 and new recommendations in Europe (2012) are shown in Table 2. The difference between these recommendations are as follows:

- variability of the treatment of *H. pylori* infections in regions with low (<20%) and high (>20%) prevalence of *H. pylori* strains resistant to clarithromycin; Maastricht/Florence IV consensus report recommended to abandon clarithromycin in treatment *H. pylori* infections or perform previous susceptibility testing to cla-

rithromycin in regions with high prevalence of *H. pylori* strains resistant to clarithromycin.

- 2<sup>nd</sup> line treatment schemes contain levofloxacin, whereas in the older version levofloxacin was only a proposal in the 3<sup>rd</sup> line treatment;

- in Maastricht/Florence IV consensus report it is strongly recommended that the 3<sup>rd</sup> line treatment have to be based on the susceptibility testing.

According to the new Maastricht/Florence Consensus 2012, the scheme for the region with the high prevalence of *H. pylori* strains resistant to clarithromycin should be introduced in Poland. Recent studies showed that 34% of strains were resistant to clarithromycin in Southern Poland (16). According to PTG data from 2008, the resistance of *H. pylori* to antibacterial drugs used in the therapy is high in Poland and amounts to 28% to clarithromycin (primary resistance 22%, secondary resistance 54%) and 46% to metronidazole (primary resistance 41%, secondary resistance 68%) (5). *H. pylori* strains

Table 1. Treatment of *H. pylori* infections (5)

<b>The first-line treatment (one of the following):</b>	
PPI, AC (1000 mg), MZ (500 mg) – twice a day, 10–14 days.	
PPI, CH (500 mg), MZ (500 mg) – twice a day, 10–14 days.	
PPI, AC (500 mg), CH(500 mg) – twice a day, 10–14 days.	
<b>The second-line treatment (one of the following):</b>	
PPI, AC(1000 mg), MZ (500 mg) – twice a day, and TC (250 mg) – three times daily; prolonged to 14 days.	
PPI, AC (1000 mg), MZ (500 mg) – twice a day, and bismuth salts (120 mg) – four times daily; prolonged to 14 days.	
<b>The third-line treatment:</b>	
Evaluation of the susceptibility of the strains to the currently used antimicrobial agents.	
Possible introduction of levofloxacin.	
Adding a probiotic.	

PPI – proton pump inhibitor; CH – clarithromycin; AC – amoxicillin; MZ – metronidazole, TC – tetracycline

Table 2. Schemes of the treatment of *H. pylori* infections according to the new guidelines – Maastricht/Florence Consensus 2012.

	<b>Prevalence of <i>H. pylori</i> strains resistant to clarithromycin</b>	
	Low (< 20%)	High (> 20%)
<b>1<sup>st</sup> line treatment</b>	PPI + CH + AC/MZ or Quadruple bismuth therapy	Quadruple bismuth therapy or – if not available sequential or concomitant non-bismuth therapy
<b>2<sup>nd</sup> line treatment</b>	Quadruple bismuth therapy or PPI + LE + AC	PPI + LE + AC
<b>3<sup>rd</sup> line treatment</b>	Based on susceptibility testing only	

PPI – proton pump inhibitor; CH – clarithromycin; AC – amoxicillin; MZ – metronidazole, LE – levofloxacin

resistant to amoxicillin have not been found in Poland (5, 17). As a result of the frequent administration of clarithromycin and metronidazole in the treatment of infections due to *H. pylori*, bacterial strains simultaneously resistant to both drugs can be found (17–19). An increasing resistance of *H. pylori* bacteria as well as their multi-drug resistance to routinely used antimicrobial agents is a serious therapeutic problem.

Current recommendation of PTG is to consider the introduction of the scheme: levofloxacin + amoxicillin with PPI as a 3<sup>rd</sup> line empirical treatment, whereas EHSR recommended this scheme as a 2<sup>nd</sup> line treatment.

Levofloxacin (S-enantiomer of ofloxacin) is a chemotherapeutic agent of the 3<sup>rd</sup> generation fluoroquinolones with a broad-spectrum activity. The development of the resistance of *H. pylori* strains to fluoroquinolones is a result of point mutations occurring mainly at positions 87 and 91 of *gyrA* gene that encodes gyrase, a binding site for quinolones in Gram-negative bacteria (20).

The introduction of levofloxacin to the treatment schemes raises many hopes. Many studies have shown the high efficiency of treatment schemes with levofloxacin (21–24).

The lack of data on the susceptibility of *H. pylori* strains isolated in Poland to levofloxacin constituted a base for performing the research to assess the level of the susceptibility to levofloxacin of *H. pylori* strains isolated from dyspeptic patients between the years 2006–2012.

## MATERIALS AND METHODS

### Patients and clinical material

In total, 811 dyspeptic patients (429 women, 382 men), with indications to gastroscopy, were enrolled to the study between 2006–2012. Medium age of patients was 45 years (16–87 years). Patients underwent gastroscopy in selected Gastroenterology Clinic in Kraków, Southern Poland. According to the clinical diagnosis, there were two main groups of patients: First group covered individuals with peptic ulcer disease (PUD), means patients with gastric or duodenal ulceration, and second group covered individuals with non-ulcer dyspepsia (NUD) means patients with diseases of upper gastrointestinal tract other than peptic ulcer disease, e.g., gastritis, duodenitis, esophagitis.

Two biopsies were taken from each patient during gastroscopy from the antrum and the body of the stomach. In total, 210 *H. pylori* strains were isolated from biopsies. These *H. pylori* strains were

obtained from 210 patients (115 women, 95 men), including 51 patients with PUD and 159 patients with NUD. Eighty one percent of these *H. pylori* strains (171/210) were isolated from patients before the treatment of *H. pylori* infections, while 19% (39/210) strains were derived from patients after the failed therapy.

The plan of the study was approved by the Bioethical Commission of the Jagiellonian University and each patient signed the informed consent for participation in the trial.

### *H. pylori* culture and susceptibility testing

**Bacterial culture.** Biopsies were homogenized in glass sterile mortars and inoculated onto the solid medium – Schaedler agar with 5% sheep blood (bioMérieux, France) and medium, Schaedler agar with 5% sheep blood with selective supplement DENT (Oxoid, UK). The culture was carried out for 72 h under microaerophilic conditions at 37°C. The presence of *H. pylori* in the examined material was confirmed by the macroscopic appearance of colonies on the medium, the Gram-stained preparation from the culture (Gram-negative, s-shaped bacteria) and positive tests for urease, catalase and oxidase. Each *H. pylori* strain was frozen and stored at –80°C for further analysis.

**Susceptibility testing.** *H. pylori* drug-susceptibility to levofloxacin was quantitatively tested by strips impregnated with antibiotic gradient (E-test, bioMérieux, France), which enables the determination of the minimum inhibitory concentration (MIC). The testing was carried out as follows: the suspension of bacteria in 0.85% NaCl sterile solution was prepared from the culture of *H. pylori*. The density of the suspension amounted to 3.0 McFarland. The suspension was inoculated onto the Schaedler agar with 5% sheep blood. After that, strips impregnated with levofloxacin gradient (E-test) were affixed according to the manual. The plates were incubated under microaerophilic conditions at 37°C for 72 h. The strains in which the MIC value exceeded 1 µg/mL were considered resistant, according to the EUCAST recommendations (25, 26). The determination was carried out against the reference *H. pylori* ATCC 43504 strain to ensure the quality of tests.

Susceptibility testing to levofloxacin for *H. pylori* strains collected between 2006–2010 was carried out after defrosting and performing the culture of the strains, due to the unavailability of E-test with levofloxacin since 2010, whereas susceptibility testing to levofloxacin for *H. pylori* strains, which were collected between 2010–2012, was carried out

immediately, together with a determination of susceptibility to other antibiotics.

Susceptibility of *H. pylori* strains to other antibiotics/chemotherapeutics was tested according to described procedures.

Interpretations of MIC values for all described strains to all tested antimicrobial agents were made according to EUCAST Clinical Breakpoint Table for *H. pylori* (26).

### Statistical analysis

The following statistical parameters were calculated: mean values, probability,  $\chi^2$  test at the 0.05 significance level (the results in which  $p \leq 0.05$  were considered statistically significant). In cases where the expected values were less than 5, the Yates correction was used.

### RESULTS

In total, 811 patients with dyspeptic symptoms were enrolled in the study. The presence of *H. pylori* infection was confirmed in 210 cases: 115 women and 95 men, which shows that both genders were equally represented. The average age of patients was 45 years.

Two hundred ten strains from 210 dyspeptic patients were included in the study. In total, 81% (171/210) strains were derived from patients before the treatment of *H. pylori* infection and 19% (39/210) were derived from patients after the unsuccessful treatment of *H. pylori* infections.

The susceptibility of *H. pylori* strains to levofloxacin was quantitatively determined by the E-test. The obtained MIC values ranged from 0.002 mg/L to 32 mg/L. The mean MIC values amounted to 1.01 mg/L (Figure 1).

In total, the ratio of *H. pylori* strains resistant to levofloxacin amounted to 8.10% (17/210), whereas the percentage of strains susceptible to levofloxacin amounted to 91.90% (193/210). The probability of the incidence of resistance to levofloxacin among *H. pylori* strains isolated from patients who were included into the trial was low and amounted to  $P(A) = 0.081$ .

In the group of 17 resistant to levofloxacin *H. pylori* strains, there were more strains isolated from women than from men (11 and 6; respectively).

Among the group of *H. pylori* strains isolated from patients before the treatment, 5.85% (10/171) of *H. pylori* strains were resistant to levofloxacin,

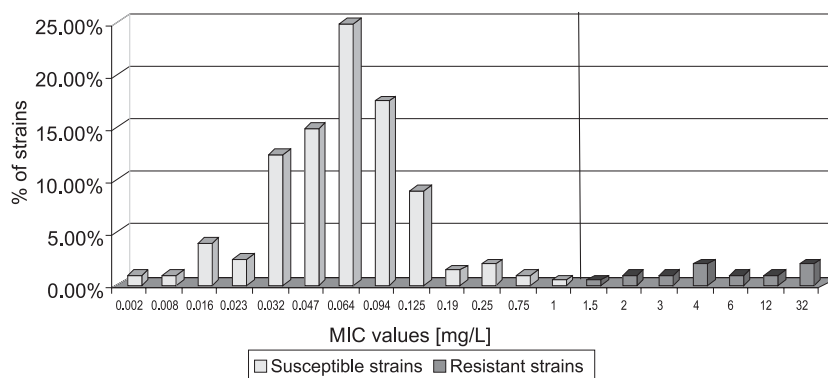


Figure 1. Activity of levofloxacin against *H. pylori* strains expressed by MIC values; MIC > 1 mg/L for resistant strains

Table 3. Comparison of *H. pylori* resistant strains isolated from patients before treatment and after failed therapy to levofloxacin (LE) out of 210 clinical *H. pylori* isolates.

Antimicrobial agent	No. (%) of resistant strains			p*
	All strains n = 210	Strains from patients before treatment n = 171	Strains from patients after failed therapy n = 39	
LE	17 (8.10%)	10 (5.85%)	7 (17.95%)	<b>0.0297**</b>

\* p (test  $\chi^2$  with Yates correction; the value  $p = 0.05$  was deemed statistically significant;

\*\* significant differences between *H. pylori* strains resistant to levofloxacin from patients before and after treatment

Table 4. Co-existence of *H. pylori* strains resistant to levofloxacin and other antibiotics.

<i>H. pylori</i> resistant to:	No. of resistant <i>H. pylori</i> strains
LE	6
LE + CH	1
LE + MZ	2
LE + CH + MZ	8
All	17

LE – levofloxacin, CH – clarithromycin, MZ – metronidazole

whereas in the group of strains isolated from patients after the treatment 17.95% (7/39) of strains were resistant to this quinolone. The statistical analysis was performed in order to check if differences between percentages of *H. pylori* strains resistant to levofloxacin from patients before and after the treatment were significant and this was the case ( $p = 0.0297$ ) (Table 3).

Moreover, the analysis of the coexistence of resistance to levofloxacin and other antibiotics was conducted. Six from 17 strains were only resistant to levofloxacin and no other antibiotics. Eleven strains were resistant to more than one antibiotic (Table 4).

## DISCUSSION

An increasing resistance of *H. pylori* strains to currently used antibiotics and chemotherapeutics is a serious therapeutic problem. A properly selected treatment model is very important as it enables *H. pylori* eradication, decreases the risk of drug-resistant strains occurrence and increases the probability of the successful therapy. Considering the high resistance of *H. pylori* strains to clarithromycin in Poland, according to Maastricht/Florence Consensus, levofloxacin should be introduced into the basic therapeutic schemes, as most of *H. pylori* strains are susceptible to this fluoroquinolone (1). An introduction of levofloxacin to the treatment regimens provides new possibilities to achieve effective *H. pylori* eradication.

The results of the study confirmed that substantial majority (91.90%) of isolated *H. pylori* strains are *in vitro* susceptible to levofloxacin. Furthermore, the low probability of incidence of *H. pylori* resistance to levofloxacin ( $P(A) = 0.081$ ) was demonstrated. The results revealed that in the Małopolska region (Southern Poland) the resistance of *H. pylori* to levofloxacin is low and rather rare (8.10%). Therefore, an introduction of this fluoro-

quinolone to the therapy of *H. pylori* infections may raise the rate of *H. pylori* eradication. Nevertheless, it is worth seeing that in the group of patients after the failed therapy, the percentage of resistant strains is statistically higher than in the group of patients before the treatment (17.95% vs. 5.85%;  $p = 0.0297$ ), however, it is still lower than the resistance to clarithromycin and metronidazole. In this respect, an introduction of levofloxacin to the 2<sup>nd</sup> line therapy seems to be a good solution. Moreover, it has been demonstrated that most *H. pylori* strains resistant to clarithromycin and metronidazole retain their susceptibility to levofloxacin (27, 28). Nonetheless, as shown Table 4, our study revealed the occurrence of *H. pylori* strains resistant to levofloxacin and clarithromycin or metronidazole or both these drugs. Therefore, it should be stressed the necessity of monitoring the resistance of *H. pylori* strains.

It should be underlined that the percentage of strains resistant to fluoroquinolones differs depending on the geographic region. It ranges from 5.3% in Iran (29), 14.3% in Japan, 16.8% in Belgium (25), 17.0% in Brazil to 22.1% in Germany (30).

The study performed by Zullo et al., in Italy in 2007 on 246 *H. pylori* strains isolated from dyspeptic patients showed that 19.1% of strains were resistant to levofloxacin (30). The study demonstrated an increase of the resistance to levofloxacin in that region, as Gatta et al., had reported the ratio of 14% of *H. pylori* strains resistant to levofloxacin earlier (in 2005) (24). The resistance increases also in other countries, e.g., in France it rose from 3.3% in 1999 to 17.5% in 2003 (25). The problem of an increasing resistance of *H. pylori* to quinolones is an effect of their frequent use in the treatment of many diseases, for instance those affecting airways or the urinary tract (31–33).

Results of many clinical trials have confirmed the effectiveness of levofloxacin in the therapy (27, 28, 34–36). Levofloxacin may constitute a good alternative in the case of double resistance of *H.*

*pylori* strains to clarithromycin and metronidazole as these strains retain susceptibility to levofloxacin (23, 27, 37).

Gatta et al. showed that the administration of levofloxacin with amoxicillin and PPI for 10 days as a third-line therapy affects the curability in over 80% (24). Gispert et al. obtained *H. pylori* eradication in 60–66% using the same therapeutic scheme, whereas Zullo reported eradication of 83% (38, 39).

In Spain, side effects and the effectiveness of the treatment of *H. pylori* infections were examined by using levofloxacin combined with amoxicillin and PPI as the first-line therapy. The study was carried out in comparison to the control group where PPI, clarithromycin and amoxicillin were administered. It was shown that the tolerance to levofloxacin was good. However, in the trial group, *H. pylori* eradication was lower as compared to the control group (71.8 and 75.5%, respectively) (40).

Nista et al. compared the effectiveness of *H. pylori* treatment between the following regimens:

- group 1: PPI, clarithromycin (500 mg), amoxicillin (1 mg),
- group 2: PPI, clarithromycin (500 mg), metronidazole (500 mg),
- group 3: PPI, clarithromycin (500 mg), levofloxacin (500 mg).

The rate of *H. pylori* eradication in these groups amounted to 75, 72 and 87%, respectively. Thus, the use of levofloxacin combined with PPI and clarithromycin gave better results than typical first-line therapeutic schemes (21).

The use of levofloxacin provides new treatment opportunities as the drug shows good tolerance and gives good therapeutic effects. Levofloxacin can be used as an alternative to amoxicillin in individuals sensitive to penicillins (34). Moreover, levofloxacin gives a higher curability rate in comparison to other therapeutic schemes, such as the standard 2<sup>nd</sup> line therapy – the quadruple therapy containing bismuth salts (41). The administration of a levofloxacin-based triple therapy for 10 days is more effective than the quadruple therapy containing bismuth salts used for 7 days (32).

## CONCLUSION

The introduction of the 2<sup>nd</sup> line levofloxacin-based triple therapy is a good alternative in the treatment of *H. pylori* infections, especially in regions with high prevalence of *H. pylori* strains resistant to clarithromycin, like Poland. Nevertheless, a need for the local national resistance

monitoring of *H. pylori* resistant strains should be stressed, because of the possibility of acquiring fast resistance to levofloxacin.

Moreover, it should be considered if triple therapy with levofloxacin will be better solution of 1<sup>st</sup> line treatment in region with high prevalence of *H. pylori* strains resistant to clarithromycin and unavailability of bismuth, instead of quadruple non-bismuth therapy that consists clarithromycin.

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